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MECHANISM OF TUMOR GROWTH IN CROWNGALL

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The ultimate cause of cell proliferation in crowngall is the Schizomycete *Bacterium tumefaciens* Sm. and T., as set forth elsewhere (1, 2, 3);[1] but the mere mechanical irritation due to the presence of a few rod-shaped bacteria in the tissues can not be the direct cause of the proliferation, since other species of bacteria either have no specific action when inoculated into plants or some quite different action, such as a wilt of the foliage following an excessive multiplication of bacteria in the vascular system, as, for example, in the disease of cucurbits due to *Bacillus tracheiphilus*, or a soft rot of shoots and tubers following an excessive multiplication of bacteria in the intercellular spaces with solvent action on the middle layers of the cell wall, as, for example, in the potato rot due to *Bacillus phytophthorus*. In each of these types of plant disease (tumor, wilt, and softrot), the ultimate cause is a bacterial infection; but the immediate or proximate cause of the phenomena, except perhaps in case of vascular embolisms, must be the chemical or physical action of enzyms or other substances produced or activated by the bacteria as a result of their metabolism, with a corresponding *reaction* on the part of the plant.

For a long time I have tried to think out the rationale of what goes on in the cell following the introduction of the crowngall organism. What we see is excessive and abnormal multiplication of the tissues resulting in a tumor, a hyperplasia. What we would wish to know is the mechanism of the growth—that is, the chemical or physical stimulus behind the observed phenomena—since, if we can comprehend it in the plant, we may be able to apply our knowledge to the understanding of similar phenomena in man and animals.

In this paper, therefore, I have ventured to offer some suggestions (supported by experiments) as to the proximate cause of the abnormal

[1] Reference is made by number to "Literature cited," pp. 185–187.

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cell proliferation in crowngall—a proliferation in opposition to the needs and well being of the plant and one following the general law of development of animal neoplasms, as I have shown elsewhere (4,18). It is believed that the experiments here detailed have important bearings not only on the origin of crowngall and of other tumors in plants, including those due to nematodes and to gall flies, but also on the origin of various animal tumors. For the most part, however, I shall here leave the reader to draw his own conclusions respecting remoter applications.

At first I conceived of the crowngall phenomena as much more complex than it really is. I thought of endotoxins and other complex substances of unknown chemical structure as probably the cause of the excessive cell proliferation, not, however, entirely excluding simpler substances, such as ammonia compounds.[1] Absorbed in other features of the investigation, I limited myself for a long time to turning over in my mind various phases of the problem with what help I could obtain from literature, but in June of last year (1916), having reached, theoretically, what seemed to me to be dependable and necessary conclusions, I began to make simple experiments, some of which have proved of great interest, and the more important of which are here first described, though I called attention to them briefly in October (5, 6).

In the beginning I had what I now believe to be a wrong conception of growth. I looked upon it as something that an outside substance could directly stimulate into development, but probably it is not that (Weigert, Ribbert, Loeb). Growth is the normal function of cells. They are always multiplying when they are not inhibited by one thing or another. Growth, then, if this view is correct, comes about not by the direct application of stimuli, but indirectly *by the removal of various inhibitions.* Under normal conditions the physiological brakes are on at all times, more or less, in both plants and animals, and only when they are entirely or largely removed in particular areas do we observe an unlimited cell proliferation resulting in the hasty and peculiar growths known as neoplasms or cancers. What, then, removes the normal growth inhibitions? And what are these inhibitions? Cold is one source of inhibition, insufficient oxygen and drouth are other causes of inhibition, but we have to do with none of these inhibitions or their opposites, nor with any others in so far as they act on the plant as a whole. The inhibition remover we are in search of is *one that acts locally, disturbing tissue equilibriums within limited areas.*

When all other conditions are favorable, such as abundant food supply, water supply, air supply, and right temperature, cell multiplication may still be held in check—that is, in unfertilized eggs—as Jacques Loeb has

[1] In a previous paper (1, p. 175), ammonium acetate is especially mentioned as a possible cause of tumor stimulus.

shown, by the surface tension of the cells. This is due, he thinks, to lipoids or fatty bodies present in their surface membrane. If one changes the surface tension of egg cells by the introduction of alkalies or fatty acids or even by punctures, growth takes place in the absence of fertilization, and a new animal (the fatherless frog or sea urchin) results. This is the problem of starting the normal physiological growth of a whole organism from an egg in the absence of sexual impact and it has been solved by withdrawing water from the surface of the egg cell. This has been done in some species by pricking the eggs, and in others by increasing the osmotic pressure of a surrounding fluid. In malignant tumors the problem is one of explaining a continuous and excessive *local growth*, abnormal in that it is outside of physiological control, but it is, I believe, a problem to be solved in the same way, viz, by showing that the overgrowth is due to a removal of the local growth inhibitions by local increase of osmotic pressures. As I now see it, tumor growth is not at all a chemical phenomenon, but rather a purely physical one, a matter of varying molecular vibrations, a development dependent on locally increased osmotic pressures which cause a movement of water and foodstuffs into the affected area. In crowngalls the removal of growth inhibitions is brought about, I think, by the physical action of substances liberated within the tumor cells as the result of the metabolism of the imprisoned bacteria. Occasionally in this paper I have used the convenient word "stimulus" in an unqualified way, but by it I mean always and only *the remover of an inhibition*.

If the cell proliferation in crowngall is due to substances liberated within the cell by the parasite, as it seems reasonable to suppose, they must be substances either identical with or at least not differing greatly in their physical or physiological action from those acting on the non-parasitized cell during normal growth and division. Of this there can be little doubt for several reasons and especially for the reason that there is no evidence of chemical injury either in the tissues surrounding a crowngall or in the tumor cells themselves, since they grow and multiply with a rapidity only to be compared with that of the cells of normal young tissues. This at once removes from consideration all actively poisonous (killing) substances and greatly simplifies the problem.

Various weak (dilute) poisons are known to cause cell proliferations in plants—that is, copper salts (7, 8), vaseline and paraffin oil (9),[1] salts of

[1] Schilling's interesting paper (9) was received at the Department of Agriculture in November, 1915, but I did not know of its existence until October of the following year—i. e., after my own experiments were completed, except for a few repetitions and some tests with poisons in great dilution. His results (numerous large-celled intumescences) were obtained by vaselining or paraffining the surface of twigs of various species: Aesculus, Sambucus, Syringa, Artocarpus, Philodendron, Ribes, Spirea, etc. Since this note was in type I have discovered that Schilling's work is only a repetition and extension of work published in 1910 by the Russian botanist, P. Wisniewski (22).

lithium (10, 11),[1] and the excretions of the larvæ of gall flies, of certain nematodes, and of various fungi. Other substances are known to cause cell proliferations in animals—that is, Sudan III and scarlet-red (12), repeated applications of tar (13), excretions of Bilharzia, etc. As bearing on the origin of neoplasms, these facts are extremely suggestive; but for obvious reasons chemicals which can not be supposed to occur in genuine neoplasms as the result of their own metabolism and can not be assumed to be excreted into them by parasites either actually or presumptively present, need not be considered here, however effective they may be as a cause of cell proliferation. In our search for the direct exciting cause of tumors we need consider only the excretions of known tumor-producing organisms; but since little or nothing is known concerning the growth-exciting substance, or substances, produced in animal neoplasms, or liberated in plants by the various gall-forming fungi, or by gall flies and gall nematodes, we may for the present confine our attention exclusively to the bacterial tumors of plants. This narrows down the problem to a few species of bacteria and even some of these—that is, *Bacterium savastanoi* (the cause of olive tubercle) and *Bact. beticola* (the cause of beet tubercle)—are negligible so far as the purposes of this paper are concerned, because I regard the tumors they produce as granulomas, not neoplasms. In fact, thus far in seeking for the physical-chemical origin of neoplasms I have sought only for answers to the following questions:

(1) What are the products of the bacterial metabolism of *Bact. tumefaciens*, the crowngall organism?

(2) Are any of these products capable of inducing cell proliferation when injected into the growing plant?

(3) If so, is this substance a sufficiently common product of other bacterial and nonbacterial tumor-producing parasites so that it may be regarded hypothetically as produced by all of them and consequently as the exciting cause of all bacterial, fungus, nematode, cynipid, and other vegetable galls, or must we suppose that various chemical growth excitants exist?

(4) And finally to what extent can these facts be supposed to apply to human and animal neoplasms?

In case of the crowngall organism I had to begin with the simpler substances, because I have as yet no knowledge of the more complex products of its metabolism, if any such exist, and certainly no very poisonous substances are produced in the crowngall, any more than in animal

[1] In this connection should be mentioned especially Caroline Rumbold's recently published results of chestnut-bark injections (11). With substances injected in the hope of controlling the Endothia chestnut-bark disease, and especially with lithium carbonate, she succeeded in causing numerous conspicuous islands of xylem to develop in the middle of the phloem. These caused bulges in the bark, visible externally. I have had the pleasure of examining her sections, and verifying her statements. The islands are developed from a cambium, the growth being always from the outer face of a bundle of bast fibers toward the surface of the stem. I could not satisfy myself as to the origin of this cambium from the normal cambium of the stem (Solereder), but it is plain that the occurrence of these islands of xylem in the phloem (they appear to be that rather than islands of phloem in xylem) is correlated with a scanty production of the normal wood of the season.

neoplasms (until secondary infections occur), because the tumor cells are not visibly injured except that they stain somewhat differently from normal embryonic tissues, are unduly excited and, along with greatly increased vegetative tendencies, have lost the whole or a great part of their polarity (orderly arrangement of cells) and also most of their power to differentiate tissues. The most conspicuous results of the growth of *Bact. tumefaciens* in culture media containing grape sugar and Witte's peptone are ammonia and alcohol (Alsberg, Brewster, Woodward) and an acid, said by Dr. Carl L. Alsberg, Chief of the Bureau of Chemistry, to be acetic acid in case of the Daisy strain with traces of oil or of a fatty acid, and by Dr. J. F. Brewster, also of the Bureau of Chemistry, one of the fatty acids (but not acetic acid) in case of the hop strain. These substances are produced by the bacterium in flasks of Jena glass containing only calcium carbonate, water, grape sugar, and peptone, and undoubtedly they are also produced by it within the cells of the tumor. According to Dr. H. E. Woodward, of the Bureau of Chemistry, small quantities of amins (but not trimethylamin) are produced by the hop strain—that is, primary or secondary amins.[1] Probably the organism also

[1] Since these two sets of analyses were made Dr. Alsberg has kindly had further analyses made for me of *Bact. tumefaciens* plated from Flats poplar, from Massachusetts rose, and from another Paris daisy gall. These were flask cultures grown over calcium carbonate in distilled water containing 1 per cent Witte's peptonum siccum, and 1 per cent Merck's c. p. dextrose, sterilized by discontinuous steam heat on 4 consecutive days and allowed to stand some days before inoculation. These analyses, which were made by Drs. Brewster and Woodward, at the end of 8 to 9 weeks, show, as in the case of the previous analyses of the hop strain, that alcohol, ammonia, and amins are formed and small quantities of fatty acids. Formic acid was detected in each of the nine flasks. No acetic acid was found in flasks inoculated with the daisy strain, but it occurred in those inoculated from poplar and rose. Aldehyde and acetone were also detected in each flask. No trimethylamin was found. The analyses of a second series of the hop strain were made in January on cultures somewhat older than the first series. (See page 186.)

In each instance I personally inoculated the flasks with great care, using agar subcultures made originally from single poured-plate colonies, flaming the necks of the flasks thoroughly in advance, turning them down on their sides to inoculate and keeping them open for a moment only in still air in a clean culture chamber, but even so there are always slight chances of contamination. To further eliminate these chances several flasks were used for each strain, several were held uninoculated for control, and the others were watched to see that growth came on normally for this organism. Finally agar poured-plates were made from each inoculated flask to judge of its continued purity and subcultures from each were tested out on plants and found to be infectious. The flasks, including those which were not inoculated and all of which had remained sterile, were then turned over to the chemists, who analyzed each one separately, with concordant results except as indicated below.

CHEMISTS' REPORT OF ANALYSES OF FLASK CULTURES

"Three flasks each of cultures marked Flats Poplar, Rose, and Resistant Daisy, dated October 21, 1916, were received from Dr. Smith on December 16, 1916. With these were also three control flasks which had not been inoculated.

"The flasks were opened and each worked through in order until finished, beginning December 18, the methods being as follows:

"The contents were filtered off from undissolved calcium carbonate. A small portion of the filtrate acidified with acetic acid and treated with a few drops of ammonium oxalate solution gave an abundant precipitate of Ca-oxalate showing that much calcium carbonate had been dissolved. This is true of all the flasks which had been inoculated. Those not inoculated gave no test for dissolved Ca. The remainder of the cloudy filtrate was distilled over milk of lime. Some of the distillations were done with the free flame, others at diminished pressure to determine whether the same products could be found at the lower distilling temperature (40°–50° C.). In either case the products were the same.

"Small portions of the alkaline distillate were tested for alcohol, acetone, and aldehyd, and the main portion, after neutralizing, was given to Dr. Woodward to test for volatile alkali.

"Acetone was first indicated by the formation of iodoform at the ordinary temperature when 2 drops of 10 per cent potassium hydroxid solution and sufficient iodine solution to produce a faint yellow color

liberates a small amount of carbon dioxid (4, p. 21–22). These substances, therefore, must be the first things tested out experimentally on plants subject to crowngall. On the start it seemed to me probable that ammonia, given off in small quantities within the cell by the multiplying bacteria, must be the sole determining factor in the abnormal cell proliferation, since as a diffusible stimulant it would enter cells readily, would increase the osmotic pressure, and would tend to enter into soapy combinations with the lipoids of the cell surface, thus changing the surface tension beyond the point of physiological cell restraint, whereupon cell division would take place. At the same time that ammonia and other concentrated bacterial products move outward, toward the surface of the tumor, where usually growth is most abundant, water and dissolved foods would move inward into the tumor, thus supplying copiously, especially at the periphery of the tumor, both the necessary stimulus and the substances needed for the constantly increasing abnormal growth. Minute continuous doses of other alkalies might act on the cells in the same way, but, as already intimated, it is here necessary to consider only such substances as are likely to be liberated within the cells of the tumor by the metabolism of the crowngall organism. If the substance which removes the growth inhibition is ammonia or any other compound produced by the crowngall bacterium as the result of its growth within the cell then the rate of cell division in the tumor would depend on the rate of bacterial multiplication and metabolism within the cells and on the relative juiciness of the tissues, which together would determine the rate of osmotic movement, while the rate of the bacterial multiplication

were added to the distillate. That acetone was present was confirmed by adding to a portion of the distillate an equal portion of strong potassium hydroxid solution and then a few drops of 10 per cent alcoholic solution of salicyl aldehyd. An orange red color is developed on warming to 40°–50° C. (Csonka, Jour. Biol. Chem. 1916, 27, p. 209).

"The acetone was removed from a separate portion of the distillate by aerating a few minutes at 40° C., and when there was no further positive test for acetone, alcohol was detected by means of the KOH-iodine reaction when iodoform was produced abundantly on warming to 60° C.

"The presence of aldehyd was detected by the Tollens's silver reduction and the fuchsin tests.

"Acetone, alcohol, and aldehyd in varying amounts were found in the distillates from each of the nine inoculated flasks. None of these was found in the controls.

"The residue in the distilling flask, representing about one-half the original volume, was diluted to original volume with distilled water, 25 cc 5 *N* sulphuric acid were added and the mixture submitted to steam distillation. The distillate was neutralized with barium hydroxid solution, evaporated in vacuum to small volume, and the barium salts obtained by further concentration on the steam bath. There appeared to be a mixture of barium salts present, but as the quantities were small only qualitative tests were made. Acetic and small amounts of formic acids were detected in flasks A, B, C (Flats Poplar), and E, F, G (Rose). Flasks I, K, and L (Daisy) gave no indication of acetic acid; formic acid only was detected (silver nitrate reduction).

"No nonvolatile acids were found in the acid residue on extracting with ether and evaporating. No fixed acids were found on acidifying the calcium carbonate residue in the culture flask and extracting with ether.

"(Signed) J. F. BREWSTER.

"JANUARY 5, 1917."

REPORT ON VOLATILE ALKALI

"In the alkaline distillates from flasks A, B, C, E, and F the following tests were made:

Ammonia	Present.
Amines	Do.
Trimethylamine	Absent.

"(Signed) H. E. WOODWARD."

would depend on a variety of external conditions and of interrelations and interreactions between the host and the parasite, such as darkness, sunlight, heat, cold, presence or absence of oxygen, abundant or scanty food supply and water supply, inherited or acquired host resistance, varying normal alkalinity or acidity of the tissues, inhibiting action on the bacteria of the acid by-products of bacterial growth, etc., ample to explain all the observed variations in the rate of growth of crowngalls and of all other neoplasms. If, furthermore, we take into account the various kinds and degrees of reaction on the part of the host, as we must, then this hypothesis is ample to explain also the whole tribe of benign tumors and all receding malignant growths.

It seemed to me, therefore, that ammonia or some salt of ammonia must be the determining factor in tumor growth. If so, then by injecting dilute ammonia water into growing plants one ought to obtain, not typical tumors, of course, for that involves the idea of a weak continuing action, hard to bring about experimentally, but an active proliferation of cells for a short time, corresponding to the brief action of the injected substance. Some killing of tissues should be expected at the points injected, especially from action of the stronger solutions but at a distance where there would be feebler action, and generally with weak solutions there should be no wounding or killing effect, but only a very marked cell proliferation. I reached this point in my reasoning before I made any experiments. Some things I overlooked, as will be seen later.

I was supported in these conclusions by the following facts out of literature. As a boy I read in some boys' wonder book that coffee berries, said to be proverbially slow to germinate, might be germinated overnight by throwing them into strong ammonia water. I bought some ammonia with my first pocket money, tried it, and found it to be as stated, but I held no checks. That was 50 years ago. Recently I have repeated the experiment with the same results. I have extended the experiment also to date seeds and to some others known to be hard to germinate—that is, black-locust seeds, but only with doubtful results, and even in the case of coffee seeds some germinate promptly in distilled water, so that, curiously enough, the experiment which more than any other determined the trend of these researches would have had no influence on my thinking had it been made in the first place properly—that is, with a sufficient number of controls in pure water.

Furthermore, in 1903–1905 (7, 8), Dr. Hermann von Schrenk, of the Missouri Botanical Garden, observed numerous warty proliferations on the leaves of cauliflowers which were attacked by *Peronospora parasitica* and had been sprayed with the fungicide copper ammonium carbonate, and investigated their origin with the following results:

In his experiments Dr. von Schrenk readily obtained numerous warty growths on cauliflower leaves within five days' time by spraying upon them copper ammonium carbonate. He also obtained warts by the

application of minute doses of other copper salts free from ammonia. Also in a first series of experiments using 2 per cent ammonia water (0.837 per cent of actual ammonia) and 5 per cent ammonium carbonate he obtained warts, but, as these solutions had been applied by means of an atomizer which had previously contained the copper solutions, the oligodynamic effects of copper were suspected and the experiments with the ammonia and ammonium carbonate were repeated, using brushes so as to exclude copper contamination, but then with more or less contradictory results, so that the author was in doubt as to the action of ammonia, and concludes the paragraph as follows:

> While several of the sprayed spots undoubtedly formed intumescences as a result of spraying with ammonium carbonate or ammonia, owing to the negative results of several plants it will not be possible without further tests to definitely ascribe the formation of these intumescences to the ammonia.

For this reason also, no doubt, he does not mention ammonia as a cause of intumescences in his general summary, which I quote:

> The results of the present investigation may be briefly stated as follows:
>
> 1. Cauliflower plants sprayed with copper ammonium carbonate produced large numbers of intumescences as a direct result of the spraying.
>
> 2. Similar intumescences were produced by means of weak solutions of copper chloride, copper acetate, copper nitrate, and copper sulphate when sprayed in very fine drops on the surface of the leaves.
>
> 3. The intumescences were formed in larger numbers on the lower surface of the leaves than on the upper surface of the leaves.
>
> 4. Intumescences were formed independent of soil or atmospheric conditions, so that the heat and water supply had nothing to do with their formation.[1]
>
> 5. Intumescences must be regarded as the result of the stimulating activity of chemical poisons, sprayed upon the leaf in weak solutions.
>
> 6. The stimulating activity exerted is probably due to the formation of compounds within the cells of high osmotic tensions, these compounds being either compounds formed by the copper salts with parts of the protoplast, or compounds formed as a result of a stimulus exerted, as evidenced by the presence of large amounts of oxidizing enzymes [2] [2 per cent gum guaiac test] as a result of an indirect stimulus exerted by the salts sprayed upon the leaf surface.

Rosen (14), who repeated Von Schrenk's copper-ammonium-carbonate experiments on cauliflower and extended them to cabbage with positive results on both plants, speaks only of the copper stimulus and apparently did not suspect the ammonia, but to me the presence of that very diffusible compound in suitable dilution explains in conjunction perhaps with carbon dioxid by far the larger part of the observed action.

To test out my conclusions respecting ammonia as the probable tumor stimulus, I first injected, in a preliminary way, small quantities of strong ammonia water (aqua ammonia, sp. gr. 0.90, 1 part, and distilled water 10 parts) into the young internodes of large castor-oil plants (*Ricinus com-*

[1] Excessive water supply and excessive heat are believed to cause intumescences on grape leaves. See Von Schrenk's papers (7, 8).

[2] Crowngalls also are very rich in oxidizing enzyms (1, p. 173).

munis). This strong-growing plant develops crowngall quickly when inoculated with *Bact. tumefaciens* (the hop strain) and is also well adapted to the experiments here described not only because the internodes are hollow and will hold a considerable quantity of fluid, but also because in the younger internodes, at least, the cavity is lined by living pith cells capable of astonishing proliferation when properly stimulated. The older internodes were also injected, but these generally yielded no results other than death of cells immediately in contact with the strong alkali. In the younger internodes, on the contrary, striking cell proliferations into the pith cavity were obtained repeatedly. That part of the pith which received the brunt of the alkaline action was in every case killed for the solution was much too strong; but the remoter tissues, especially the inner face of certain xylem bundles, proliferated freely, almost furiously, often for long distances, forming raised snow-white cushions of delicate cells, which in their morphology strongly suggested those cells which are to be seen growing from the margins of gum pockets, but were much more abundant and active (Pl. 4 and 5). The cell proliferations, which in this case arise from the tissues of the vascular bundle or its sheath (inner face of the xylem or of the endocycle), are apparently much more abundant than one would expect for the repair of a simple wound. These cushions have the appearance of tissues growing rapidly under an active stimulus of some sort both because of the volume of the growths visible within a given short time and because of the great number of the cells composing them.

When these results were obtained with the strong ammonia two questions arose:

(1) Whether the growths were simply unusually active wound proliferations—that is, exclusively repair cells, or were really in part proliferations due to the stimulating (inhibition-removing) action of the alkali, and not exclusively to its killing (wounding) action;

(2) Whether the same results could be obtained with the various salts of ammonia.

Respecting the first inquiry we might conceive the growth response to be due (1) to the killing effect of the ammonia, the proliferations being simply the development of wound-repair tissue, in which case one ought to be able to obtain the same results by use of many other injurious substances, and killing effects should always be visible, while greatly diluted ammonia would then have no visible effect; or (2) to a specific effect of the ammonia due to the removal of physiological (or physical) growth inhibitions, in which case it should be excessive as compared with ordinary wound-repair tissue, should be obtained with very weak aqua ammonia (high dilutions) in the absence of any wounds, and should not be obtained from the use of acids or other poisons unless they also have a specific effect on the cell membrane; or (3) a nonspecific, water-attractive

(osmosis-inducing) purely physical effect, in which case many other substances would cause it, even in great dilution, and there would be then, also, no evidence of any wounding of the tissues except in case of excessive doses.

These various inquiries formed the basis of further experiments.

First, I tested out in Ricinus stems, and in part also in young green tomato fruits the effect of the following compounds: Urea, ammonium carbonate, ammonium tartrate, ammonium citrate, ammonium malate, ammonium acetate, ammonium sulphate, ammonium sulphite, ammonium nitrate, ammonium lactate, ammonium chlorid, ammonium oxalate, ammonium formate, ammonium salicylate, ammonium succinate, dibasic ammonium phosphate, and monobasic ammonium phosphate, with generally positive results and often very striking ones, and also once in case of the last-mentioned substance with very unexpected structural transformations to be described later.

To return now to the individual action of the various salts of ammonia. I obtained in the pith cavity of Ricinus and in the interior of green tomato fruits striking proliferations with a variety of these salts, as may be seen by consulting the accompanying plates, and that too whether the tissues were or were not visibly injured. Some of these substances at first were used too strong and exerted a local killing effect, but remote from these wounds fine proliferations were obtained from the apparently uninjured surface layer of the pith in Ricinus stems and from the inner pericarp wall in the green tomato fruits. Special attention is called to the outgrowths on the uninjured inner face of the green carpels of the tomato as a result of the action of ammonium carbonate (Pl. 6, fig. 1 and 2) ammonium acetate (Pl. 6, fig. 3), and ammonium tartrate (Pl. 7, 8, 9, and 15). The relatively small size of the cells in Plates 7 and 8, as compared with the excessively large cells figured by Dr. von Schrenk from his cauliflower intumescences, need not excite comment, since in Plate 9 and also further on we shall see that the cells of the intumescences which I have produced while often smaller are also sometimes much larger than corresponding normal cells of the plant and frequently vary among themselves within wide limits, even in the same intumescence, just as they do in bacterially induced crown galls. It also appears to make a difference what chemical is used or else different volumes of the same substance act differently, large cells being formed under one stimulus and small cells under another stimulus.

Serial sections cut from green tomato fruits exposed to the ammonium tartrate show that the internal proliferations have arisen in many cases at least from a single-layered epithelium in which and under which there is no evidence of wounding. In the same way under the numerous proliferations obtained on tomato carpels with the ammonium acetate I could find no evidence of any cell injuries. In the strengths used the

tissues reacted least to the most injurious substances—that is, to ammonium oxalate, ammonium chlorid, and ammonium nitrate.

I then determined on these plants the action of various dilute pure acids, the same being distilled water solutions of the acid component of the salts already tested, viz, uric acid, carbonic acid, tartaric, citric, acetic, butyric, lactic, oxalic, formic, succinic, salicylic, sulphuric, hydrochloric, and phosphoric acids.

It would take too long to describe in detail all of the experiments with acids which consumed a whole summer and were also unexpectedly positive—that is, showed quite clearly that galls or intumescences may be caused by the action of many dilute acids, including such as various bacteria, larvæ of gall flies, and other parasites are able to produce, and that the previous response to the ammonia salts was not exclusively a response to the alkaline element in them but might be a response due to the combined or joint action of these substances on cells young enough to respond to it.

Very fine intumescences were obtained in this way by injecting 10 per cent solutions of the organic acids—that is, uric acid, malic acid (Pl. 10), citric acid, and tartaric acid. Often the whole wall of the pith cavity was covered with them, and there were frequently large outgrowths around the needle wound where it entered the pith cavity (Pl. 11). But also I obtained intumescences with sulphuric acid and with phosphoric acid and sparingly even with distilled water. These results were unexpected and at first disconcerting, it must be confessed, for I had not then read Loeb's latest remarkable book on parthenogenesis, wherein are detailed many positive and splendid results with acids on animal eggs (15), and which would have advanced my work by at least two years had I read it when it first appeared. I was looking for a specific chemical effect as tumor cause and I discovered instead a general physical (osmotic) effect as cause. I will not undertake to explain the specific action on the cell of all these various substances, since Loeb has speculated on this subject better than I can hope to, and since I have seen nothing to contradict his hypotheses. Much remains for the future, so far as an understanding of the exact mechanism of cell division is concerned, but Loeb seems to have made out quite clearly that the stimulus which sets unfertilized eggs to growing is a purely physical one—that is, an increase in the osmotic pressure of the solutions in which they are placed causes loss of water from the egg surface with the formation of a membrane, which is the beginning of cell division. I believe increased local osmotic pressure also explains the results I have obtained, as well as those which occur naturally in tumors. It is the beginning, I think, of all tumor growth.

If these things are so, why, then, does not the injection of any organism produce a tumor? This is a proper question and may be answered tentatively as follows: (1) In many cases the injected bacteria do not

grow, and therefore the products of their metabolism do not come into play; (2) most of the parasitic forms cause a loss of turgor, requisite for cell-division, either (*a*) by obstructing water-conducting channels, causing the plant to wilt, or (*b*) by excretion of plasma-killing toxins which pave the way for the bacterial advance into the tissues, or (*c*) by excretion of enzyms or other solvents of the middle lamellæ of the cell-wall, in which case we have a softrot—that is, a complete or partial disintegration of the tissues; (3) moreover, the case is not so unique as some persons suppose, because various other bacteria than the crown-gall organism do actually produce overgrowths, large or small—that is, granulomas (often of large size), tyloses in vessels or small surface intumescences (I know of half a dozen or more such bacteria and new ones are coming to light every year); (4) a very considerable number of fungi also cause overgrowths; (5) the number of insects that cause overgrowths is legion.

I will only say here, further, that there is really no reason to be disturbed by the variety of substances shown to be capable of causing cell proliferation, since probably only such substances can cause neoplasms as are thrown off locally and continuously into the tissues by cancer organisms, and that too *intracellularly*, for otherwise only granulomas should result in plants, while in animals the blood stream would quickly remove and effectually dispose of any irritating substances not continually replenished. Moreover, in animals the tissue naturally most exposed to foreign substances—viz, the epithelium—is by nature the most resistant—that is, the last to yield to cancerous proliferation.

Having come to the conclusion that I was dealing with a general physical problem rather than with a specific chemical problem, I next tried the effect of injecting various foods and poisons as follows:

Ten per cent tannic acid (Pl. 11), 10 per cent ethyl alcohol, 2 per cent sodium chlorid, 2 per cent sodium carbonate, 5 per cent sodium bicarbonate (Pl. 12), *N/20* sodium hydroxid, 5 per cent ammonium bicarbonate (Pl. 13), clear lime water (Pl. 14), milk of lime (caustic), 1 to 10,000 mercuric chlorid, 0.5 per cent carbolic acid, chloroform water, 1 to 1,000,000 copper sulphate water, 5 per cent grape sugar (Pl. 14), 5 per cent cane sugar (Pl. 15).

These experiments also yielded positive results and very striking ones in some cases, even the grape-sugar and cane-sugar ones.

Next, I undertook to determine the effect of very minute doses of ammonia—doses so weak that surface woundings were not to be expected. For this purpose I used the feeble alkaline vapors arising from dilute solutions of urea, ammonium carbonate, and the two ammonium phosphates (Pl. 16 and 17), these substances being inclosed in open small test tubes sealed into the pith cavity of Ricinus stems by means of collodion or surgeons' adhesive strap. I also injected 1 to 100 and 1 to 1,200 ammonia water with striking results (Pl. 18–31). Subsequently

I inserted tubes containing stronger solutions of monobasic ammonium phosphate (20 per cent) and from its vapors obtained many positive results (Pl. 32–36). All of these experiments yielded positive results—that is, fine proliferations from surface cells of the pith cavity, *not only in the opened internodes, but also in many unopened ones above and below*, and consequently where the action had to take place at a distance through thick cross walls.

From these experiments there can be no reasonable doubt, I think, that any soluble substance whatsoever, except a killing, a plasmolyzing, or an oxygen-absorbing substance, if continually liberated in excess locally in tissues would be competent to induce tumor formation.

One of the most striking results of these experiments has been *the production of a stem within a stem*. Recently in THE JOURNAL OF AGRICULTURAL RESEARCH (Apr. 24, 1916) in detailing various experiments made with the crowngall organism I showed that under some circumstances the bacterial stimulus caused the production of a stem within a stem (21, Pl. 21). The plant used was the common tobacco (*Nicotiana tabacum*), and the new stem, or more properly *stele* (which I called a "tumor strand" because at frequent intervals it induced the formation of secondary leafy tumors), was developed in the outer cortex of the normal tobacco stem between tumors where I believe no such structure was ever seen before.

I have now been able to produce this strange phenomenon of a stele within a stele in the absence of the crowngall bacterium by simply injecting one of its products—to wit, an ammonium compound—into growing tissues. Internodes of the castor-oil plant (*Ricinus communis*) were used for all of these experiments. I selected this plant rather than tobacco because it is easier to work with than a plant having a solid stem, and also because the fluids could be injected in any desired small quantity without tearing the tissues, the only mechanical injury being the small hole made by the hypodermic needle.

The results obtained have been very surprising and are, I think, quite suggestive not only of the growth previously obtained between tumors in a tobacco stem by means of the bacteria (the cortical stele formation), but also of the tumor formation itself. The photomicrographs show my results very clearly and only a few explanatory words are necessary. These results were obtained with the monobasic ammonium phosphate. Young, half-grown, and old internodes were injected but striking results were obtained only once, when a very young internode was injected.

The manner of experimentation was very simple. The needle of a glass-walled hypodermic syringe was thrust through the upper part of the wall of the internode and the cavity (very small in the young internode) was filled with distilled water in which 5 per cent of the ammonium salt had been dissolved. Sometimes the needle wound was closed with collo-

dion, but this is not necessary. Needle wounds without injection—that is, where only air entered, gave no such results; neither did the injection of pure water. The bark and wood cylinder were not visibly affected by the injection—that is, there was no change in color and no distortion or abnormal increase in the diameter of this part of the stem—but the pith, in contact with the solution, proliferated into the pith cavity enormously as a compact cylinder of small cells narrowing the lumen almost to closure, especially at the upper end, the diameter of the stem wall being increased to more than double its normal thickness—that is, from 3 to 7 mm. The normal Ricinus stem of this age contains a large pith cavity and a relatively thin wall. The xylem-phloem cylinder in such a stem is well toward the surface, the cortex being thin and the pith relatively thick. (Pl. 37, fig. 1.) The bundle is not bicollateral.

Adding the proliferated pith tissue to that normally present gives a very thick cylinder of pith in the inner part of which, entirely surrounding the pith cavity (source of the stimulus), a second xylem-phloem cylinder has developed. (Pl. 37, fig. 2.) About the tenth or twelfth day death occurred in the middle part of the thickened pith cylinder as shown by the appearance on cross sections (Pl. 37, fig. 2) of a wide white ring midway between the two complete xylem-phloem cylinders, indicating substitution of air for water, and soon after the inner xylem-phloem cylinder was torn from the outer part of the pith, probably by cessation of growth on the part of the inner pathological cylinder, the stimulus from the pith cavity having been exhausted, with continued growth of the outer xylem-phloem cylinder. The inner cylinder then lies loose in a large cavity, like the skin of a caterpillar in a cocoon, or like a rough-coated stick of macaroni (Pl. 37, fig. 3), the surface being covered with coarse shreds of the dead, white pith. The interior of this cylinder is still living and the inner face is covered with a sheet of living, loosely connected, rounded, glistening, turgid cells—the final proliferations in response to the waning stimulus. The wall of the outer inclosing stem has now about the same total thickness and relative arrangement of tissues as the whole stem had at the beginning of the experiment (compare fig. 2, 3, and 4 of Pl. 37) except that the pith is narrower (approximately one-fifth cortex, two-fifths xylem-phloem, two-fifths pith); but its inner face is covered with coarse white flecks of dead pith and all its inner cells are more or less shriveled. The pathological stele in the tobacco stem occurs in the outer part of the fundamental connective or conjunctive tissue (the cortex); and this pathological stele in Ricinus occurs in the pith, another part of the same fundamental tissue.

This second xylem-phloem contains primitive spirals, tracheæ, wood fibers, cambium, and sieve-tube tissue, the numerous bundles that compose it being regularly separated by medullary rays. (Pl. 38–41.) This pathological stele within the normal stem has a reversed polarity—that is, its spiral vessels face those of the normal xylem cylinder but are

separated from them by a wide tract of pith, while its phloem faces the pith cavity but does not come to its surface. Within itself the tissues of this abnormal xylem-phloem cylinder are arranged in a fairly orderly manner, but not as perfectly as in the normal outer xylem-phloem cylinder—that is, (1) there is an increased number of bundles, (2) the conjunctive tissue between the bundles is narrower and its component cells are smaller than in the normal xylem-phloem cylinder, (3) the cambium ring is thicker, (4) the xylem-phloem vessels are fewer, and (5) the wood fibers are more numerous. There are also many distortions strikingly suggestive of crowngall—that is, twisted bundles, spiral vessels, and tracheæ lying diagonally or at right angles to the longer axis of the stem, and loops and distortions of the cambium (toward the pith cavity) leading in places to the formation of islands of xylem-phloem (well-staining tracheæ and occasional sieve tubes) inside the second xylem-phloem cylinder close to the pith cavity (Pl. 39–41), as if the plant when cut was in process of constructing other xylem-phloem cylinders more imperfect than the second because the stimulus was exhausted. The tissues in the imperfect third cylinder face the reverse way from those of the second cylinder, while those in the fourth face the same way. Beyond these is also a cambium without accompanying vessels (broken dark line, Pl. 38, fig. 1).

Great thickenings of the pith with the same cylindrical tearing loose of the inner (proliferated) part from the outer (normal) pith were also obtained with ammonia (Pl. 29, fig. 1), with dibasic ammonium phosphate (Pl. 42) and with other substances, but no perfect double cylinder of xylem-phloem. I did obtain, however, in a young internode next above an injected one ($\frac{1}{100}$ ammonia) a dislocation of a few vascular bundles extending the entire length of the internode. These bundles are buried in normal looking pith (Pl. 20) which forms a very decided ridge projecting into the pith cavity. Here the general appearance strongly suggests the pathological stele obtained in the tobacco stem, but the orientation of the xylem-phloem is exactly the reverse of that in the tobacco stele. In that there was an outer hollow cylinder of phloem, a middle hollow cylinder of cambium and an inner cylinder of xylem. In this Ricinus stele the outermost hollow cylinder is xylem, the middle hollow cylinder is cambium, and the central solid cylinder is phloem (Pl. 43). If, however, we consider that part of each stele which faces the normal xylem-phloem cylinder they are alike—that is, they follow the general law of duplication of parts—viz, that the excessive part is a mirror image of the normal part.

Moreover, the structure of these dislocated bundles is curiously like the whorls of tissue (tracheæ, etc.) that occur in the tumor-strand or fundamental tissue in the interior of secondary crowngalls produced by *Bact. tumefaciens*. I have frequently figured (4, Pl. 36, 39, 40, 83, 84; 18, Pl. 8, 12, fig. 48) and referred to these interesting whorls, but have

not hitherto described their component parts. They are within the vascular cylinder of the tumor in what I take to be modified pith and they also have the same order and arrangement of parts as the Ricinus steles just described—viz, spiral vessels on their periphery followed by tracheæ and cambium within which are sieve tubes, and sometimes in the larger ones a small amount of the fundamental tissue forming a coarse-celled center.

I observed the same phenomenon in another Ricinus stem the pith cavity of which had received ammonia. Here no less than eight concentric medullary bundles (phloem strands surrounded by xylem, as in crowngalls) developed in the outer pith near the vascular cylinder (Pl. 44 and 45) at one level and 16 at another level in a circumference of less than 1 cm. In this connection, however, see Plate 65.

Furthermore, in studying the outer part of the same cross sections (middle part of the Ricinus internode shown on Plate 5) I discovered under the dead pith cells at either side of the proliferating cells, shown in the center of the picture, cushions of proliferating tissue derived from the inner (parenchymatic) face of the uninjured xylem bundles and in the innermost part of these cushions close to the layer of killed tissue a small group of phloem cells under which (that is, farther from the pith cavity) are spiral vessels, with what appears to be cambium between them (Pl. 46). In other words, here is another example of a pathologically induced second xylem-phloem cylinder, much more imperfect than that produced by the ammonium phosphate but oriented in the same way—that is, it is the reverse or mirror image of the normal xylem-phloem, but is separated from the latter by a considerable abnormal growth of parenchyma. In Plate 47, figures 1 and 2 show cross sections of the central strongly proliferating part of Plate 5. Only tissues near its outer part are here shown, but in them are tracheæ and twisted cells strongly suggestive of crowngall distortions, while deeper in (Pl. 45, fig. 1) are the normal elements of the bundle. Calcium-oxalate crystals are very numerous in and near the killed pith tissues, are not elsewhere present in the sections, and have never been seen by the writer in normal Ricinus pith of this age. This suggests that normally in dividing cells ammonia is perhaps the first term in a series of reactions leading, in the plant, to the production of oxalic acid, which, as we know, is an almost universal product of its growth.

In one case also where limewater was injected I succeeded, in the lower third of an internode, in closing the pith cavity altogether with normal pith proliferations in which no wound tissue is visible (Pl. 14, fig. 3), but no second cylinder of xylem-phloem was formed in it; nor have I thus far succeeded in reproducing the phenomenon with the monobasic ammonium phosphate. My later experiments, I now believe, were done on internodes which were too old.

These results and recent ones with crowngall teratoids (bacterially induced embryomas) lead me to believe that not only the origin of fasciations but also of many other duplications is to be sought in local and feeble infections by a variety of deep-seated microorganisms.

Duplicate vascular cylinders in the pith and scattered concentric cortical or medullary bundles do not occur normally in the internodes of Chrysanthemum, Nicotiana, Ricinus, or any of the higher plants I have worked with, so far as I have observed, but triple sheathing cylinders of xylem-phloem (the inner ones standing in pith) occur normally in some of the ferns (Matonia). Scattered concentric medullary bundles (with a central phloem) occur in the stems of various dicotyledons, but are regarded as anomalous, while concentric cortical bundles are said to be rare (Solereder). Some of the phenomena here described are therefore possibly to be regarded as in the nature of reversions to earlier and more primitive stem structures or else as indications of premature development of tissues which are normally developed only toward the end of the life cycle of the plant. Since this paragraph was written I have found a paper by Martin Möbius (25) describing and figuring a concentric bundle with central phloem from the axis of inflorescence in *Ricinus communis*. This stimulated me to examine sections from numerous nodes and internodes of Ricinus, as well as from the tap root, hypocotyl, cotyledons, leaves, and axis of inflorescence with the following result:

Concentric medullary bundles with phloem at the center are very numerous in the axis of inflorescence (Pl. 65, fig. 1); they occur sparingly in the nodes, and are present in branch-gaps immediately above the node (Pl. 65, fig. 2). I did not find them elsewhere in the internodes. They were not seen in the taproot, hypocotyl, cotyledons, petioles, or leaf-gaps.

Möbius gives reference to earlier literature on concentric bundles of this type, especially Russow (23) and Bergendal (24), and at the end of his paper a list of families in which they have been observed.

I have not been able to find such bundles in any part of the Paris daisy, although they occur in the crowngall on this plant and normally in the pith of certain compositæ (*Scorzonera hispanica* and *Tragopogon pratensis*).

Finally, I repeated Dr. von Schrenk's ammonia tests on cauliflower, obtaining numerous magnificent proliferations which could only be attributed to the ammonia used, since no copper salts entered into the experiments. At first I painted on dilute ammonia, but later and much more satisfactorily (Pl. 48, 49) I exposed the plants for a few minutes in a tight box containing 10½ cubic feet of air space to vapors from a small quantity (1 c. c., ½ c. c., $\frac{1}{10}$ c. c.) of aqua ammonia (0.90 sp. gr.). The vapor undoubtedly entered through the stomata which are chiefly

on the under surface, and the outgrowths are almost entirely from the loose parenchyma of the leaf (Pl. 50, 52). In structure these tumors are either pure hypertrophies or mixed hyperplasia and hypertrophy (Pl. 50–54).

Also, I obtained numerous fine intumescences on cauliflower leaves by exposing them for a short time to mixed vapors of ethyl alcohol and acetic acid (Carnoy's fixing fluid), but only when the stomata were nearly closed (presumably). In maturer rapidly transpiring leaves on the same plant the effect of the vapor was entirely different—that is, there was a localized killing effect, giving to the leaves a curious mottled green-and-white appearance (Pl. 55). I looked in vain on such leaves for intumescences. There was not a trace even on the margins of the white spots. These leaves were in full growth and vigor, not old leaves. Those leaves which developed the intumescences were younger leaves, only partly developed at the time of exposure (Pl. 56). These would have their breathing pores closed or nearly closed at noon, and consequently only a stimulating minimum of the vapor could enter. The appearance of these growths in fresh vertical sections is shown on Plate 57 and in cross and vertical sections from fixed and stained material on Plates 58 to 61. They are quite unlike most of the ammonia intumescences in structure, although derived in good part from the same tissues of the leaf (loose parenchyma)—that is, the ammonia intumescences, except two old ones, are made up almost exclusively of a few hypertrophied cells (Pl. 52–54), while these are composed of many intermingled small to moderately coarse cells, much more suggestive of a crowngall hyperplasia, than which, however, they are more regular. They also suggest incipient shoots—that is, growing points, and those 7 days old show the beginnings of a vascular system derived from the smaller vascular bundles of the leaf (Pl. 60, 61). I believe they are actually pathological growing points, and if so they are a striking example of the ability of the cauliflower leaf to develop totipotent tissues (embryomas) from many parts of its under surface when properly stimulated. The cells of these intumescences form a compact tissue that is very unlike the loose parenchyma from which it has been derived. There is, therefore, a suggestion in these results that one chemical substance may induce hyperplasia of cells and another hypertrophy, both of which phenomena occur in crowngall and in various animal neoplasms. Still other substances are perhaps required to produce multinucleate cells.[1]

[1] As distinguished from hyperplasia three striking instances of hypertrophy of cells in connection with tumor growths may be referred to here: (1) The root nodule of legumes due to nitrogen-fixing bacteria, (2) the finger and toes of turnips and cabbages due to a spore-producing myxomycete, (3) galls on the roots of a great variety of plants caused by parasitic nematodes of the genus Heterodera. In the first and second cases the enormously enlarged cells are densely occupied by the parasites which destroy the cell contents, plasm and nucleus, and consequently these cells are not giant cells in the meaning of the animal pathologist, namely, not multinucleate cells. On the contrary, the enormously enlarged cells in the nematode galls should be of special interest to animal pathologists, because they are true giant cells, their development

Similar results were obtained on cauliflower leaves by exposure to the vapor of secondary methylamine (Pl. 46, 62, 63, 64).

Also on cauliflower plants exposed to the vapor of primary ethylamine the younger leaves showed distortions and thickenings of the parenchyma with changes in the palisade tissue quite suggestive of curly top of sugar beets, mosaic of tobacco, and similar diseases of undetermined origin but suspected of being due to parasites (Pls. 63, 64). The etiology of these obscure diseases can perhaps be unraveled by exposure of healthy plants to the products of a variety of microorganisms and here, possibly, is a clew.

Consequently without entering into further details I will say that I think I have established my original hypothesis—viz, that dilute ammonia causes intumescences and have rendered it probable that ammonia liberated within the cell in small quantities by the imprisoned bacteria must be one of the causes of the excessive and abnormal cell proliferation in crowngall. Probably amin compounds also help to determine it. Since an acid and an alcohol are likewise produced by the crowngall bacteria and this alcohol and this acid (as well as many other acids) in pure dilution and also in combination with ammonia caused galls or intumescences in my experiments, the acid (or acids), the alkalies, and the alcohol must, I believe, act together in producing the tumors, and *osmotically rather than chemically.*

To return now to the experiments as a whole, here is abundant further evidence that changes in stimulus can produce changes in structure, if any such additional evidence were needed, considering the multiplicity of arguments from regeneration experiments, especially on animals. And this time the changes, some of which if more regular (and there is a reason for that) are nevertheless as striking as anything observed in beginning neoplasms, have been produced, in the absence of bacteria, with known chemical substances—some of them the by-products of the metabolism of a cancer parasite, provided, of course, that crowngall is admitted into the family of the neoplasms, as I think it must be (18, 19, 20).

out of ordinary cells being in some way brought about by a closely attached but external parasite which feeds upon them as they grow by thrusting its mouth parts into them. These cells are often 20–30 nucleate and a hundred times as large as the normal cells, so that often in sections of the galls they have been mistaken for parts of the parasite, being quite unlike anything ever observed in the normal tissues of the host. Here the stimulating substance, which might well be some ammonia compound from the urine or feces of the feeding larvæ, not only stretches the cell wall, as in 1 and 2, but that or some other substance also causes a fission of the nucleus. But fission of the nucleus may occur in crowngall without any marked enlargement of the cell, and also cells in that tumor may show hypertrophy without developing multiple nuclei. When discussing my results with Dr. N. A. Cobb, of the Bureau of Plant Industry, he called my attention to certain facts respecting the orange-root nematode of Florida, which seem to me to be very pertinent in this connection. This eelworm, which is the type of his new genus Tylenchulus (16, 17), does not produce galls, although it is closely related systematically to the gall-forming eelworms of the genus Heterodera. This striking difference is correlated with the fact that the larvæ of Tylenchulus are hatched free in the earth and never insert into the roots of the orange plant anything but their head parts, all their anal excretions being voided into the earth, whereas the larvæ of Heterodera are entirely buried in the plant from the beginning, so that all their excretions must necessarily come into intimate contact with the growing tissues of the host. It would seem, therefore, that nematode galls must be due to anal rather than buccal or skin excretions.

No unbiased person, it seems to me, remembering that I have done these things with a fleeting stimulus (fluid or vapor) applied entirely from a cavity (stomatal, carpellary, or pith) and not acting from within the cells as would be the case in cancer, can look at the striking cell proliferations which I have obtained and which are so like early stages of crown-gall without being convinced that the growths would have gone on indefinitely, with the formation of large irregular tumors, rupturing to the surface if the chemical substances leading to the increased osmotic pressure could have been applied slowly, continuously, and in varying localities as must be the case when the by-products of intracellular proliferating bacteria are the cause of the tumor. Moreover, if wood and bark and organ fragments of all sorts (18, 21) can be developed out of place in plants by a local stimulus why not also in the same way cartilage, bone, muscle, and fœtal fragments out of place in animals?

To conclude, it would seem, therefore, that in local osmotic action (possibly in some stages chemical action also) of various substances (aldehyde, acetone, alcohol, acids, alkalies) thrown into cells and diffusing from them in various directions, as the result of the metabolism of a feeble intracellular parasite or symbiont together with the resultant counter movements of water and food supply we have, in crowngall at least and presumptively also in animal neoplasms, the explanation of tumor growth—that is, of that extensive multiplication of cells in opposition to physiological control which has so long puzzled pathologists and all students of overgrowths.

SUPPLEMENTARY REPORT OF THE CHEMIST.

[This report was received too late to be inserted in its proper place in the text. It should be read in connection with the Chemist's Report, on page 169.]

Flasks N, O, P, and Q, cultures of the hop strain of *Bacterium tumefaciens* inoculated on November 4, and flasks R and S, checks on the hop, were received from Dr. Smith for analysis.

Flasks N, O, and P were opened on January 15 (72d day), their contents united and distilled to get rid of neutral volatile products. This distillate gave a positive test for aldehyde. Alcohol and acetone were absent.

The residue being reduced to about half its original volume was treated with 75 c. c. 5*N* sulphuric acid and distilled with steam. The distillate was neutralized with barium hydroxid, evaporated to dryness and the crystalline residue dissolved in water and filtered. The filtrate was treated with zinc sulphate. This precipitated the barium as sulphate, which was filtered off. The clear filtrate containing the organic zinc salts was evaporated to a very small volume and taken up in alcohol. Zinc acetate is soluble in alcohol, while zinc formate is insoluble. A considerable precipitate showed the presence of zinc formate. This was confirmed by dissolving in water after filtering and boiling with a few drops of silver nitrate, when metallic silver was deposited as a mirror.

The filtrate was evaporated to dryness and taken up in just enough water to dissolve it. On adding silver-nitrate solution a brown precipitate formed, which turned black on boiling, indicating formic acid. Acetic acid was not found in these cultures.

Jan. 16, 1917. (Signed.) J. F. BREWSTER.

Plates were poured from each one of these flasks before turning over to the chemist and appeared to be pure cultures of the crowngall organism. Plants have also been inoculated with subcultures from colonies taken from these plates, but it is too early to know results.

The bacterium used for these flask cultures was the original hop strain kept for a long time in the laboratory without passage through plants (9 years), but was still infectious. That used for the first set of hop flasks (which were only 2 weeks old) was the same strain but after passage through sunflower in 1915.

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PLATE 4

Pith cavity of a stem of *Ricinus communis* showing proliferations of deep origin due to the injection of a mixture of strong ammonia and water (aqua ammonia, sp. gr. 0.90, 1 part, and distilled water 10 parts). The surface of the pith, as at X, X, is dead. The proliferations are from the inner face of the xylem at the bottom of the internode and the needle wound was about 5 cm. above this point. Injected on June 2. Photographed on July 13, 1916. Time, 41 days. ×14.

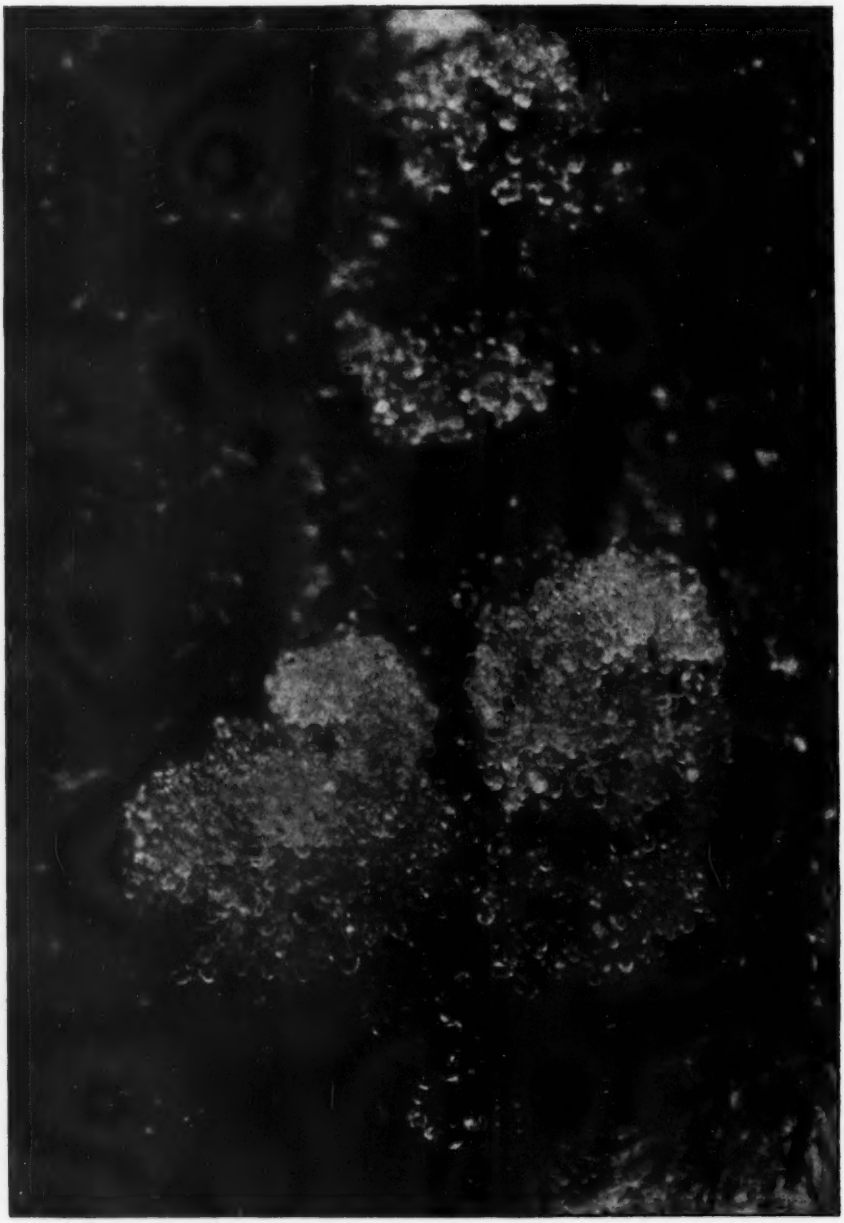

PLATE 5

Opposite side of the pith cavity of *Ricinus communis* shown in Plate 4, but more highly magnified to show the individual cells of the proliferations. ×28. For cross sections see Plates 44 to 47. The middle of this figure is about 1 inch above the node.

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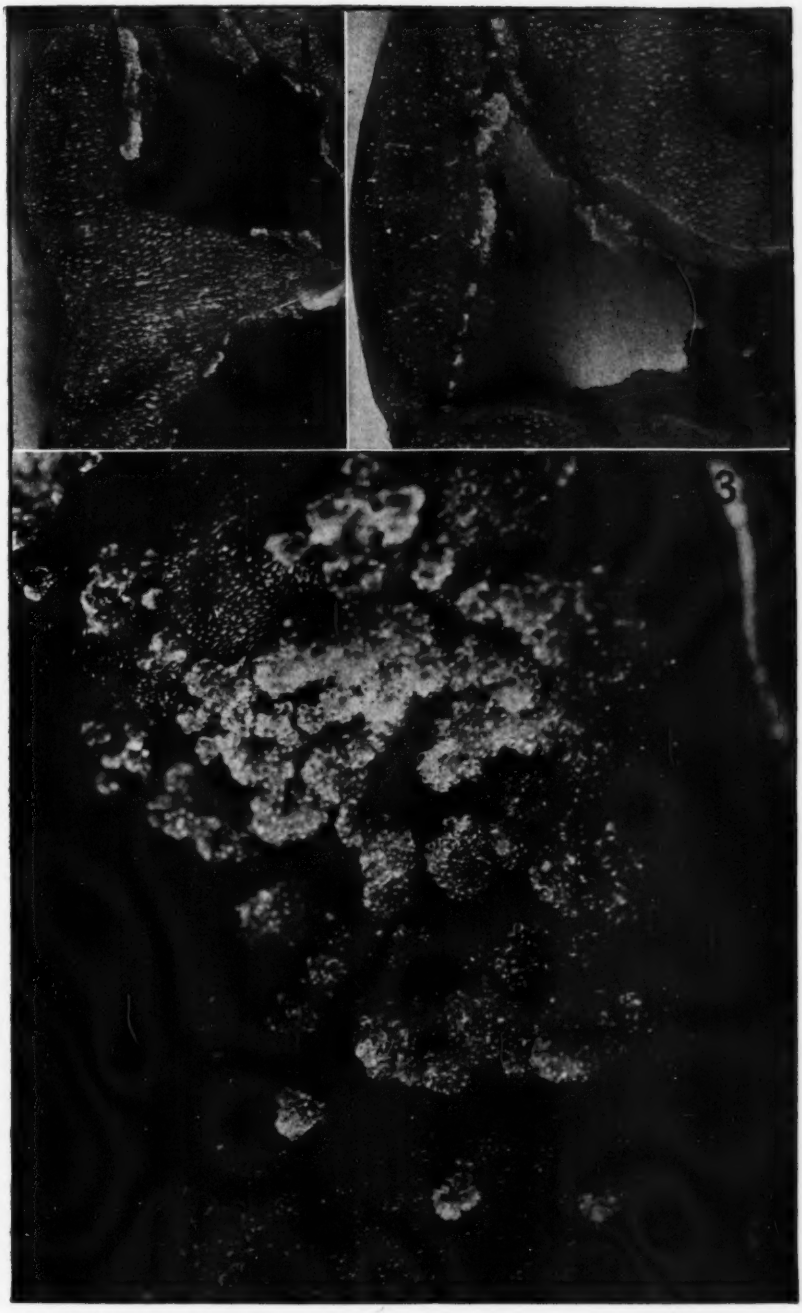
PLATE 6

1, 2.—Sections of a green tomato fruit showing carpellary surface proliferations due to ammonium carbonate (20 per cent solution). Time, 10 days. Injected on June 10. Photographed on June 20, 1916. ×7. It is probable that only vapors from the carbonate touched these places, otherwise there would have been woundings.

3.—Inner face of carpel of a green tomato fruit showing numerous mulberry-like white surface proliferations due to the injection of ammonium acetate (20 per cent solution). Carpel surface uninjured—that is, there were no dead specks in the pale green carpel wall either between these proliferations or under them. Photographed on June 22, 1916. Time, 12 days. ×14.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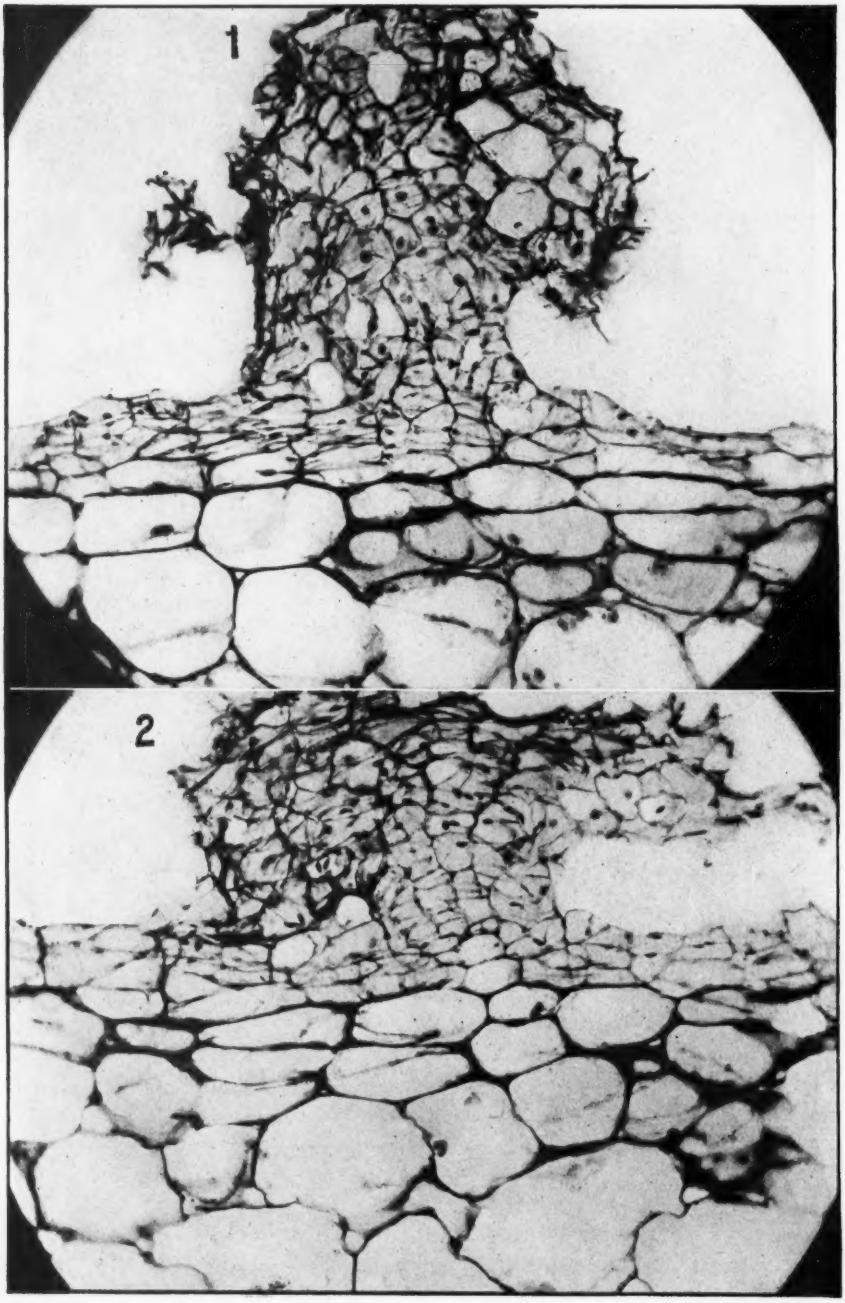
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PLATE 7

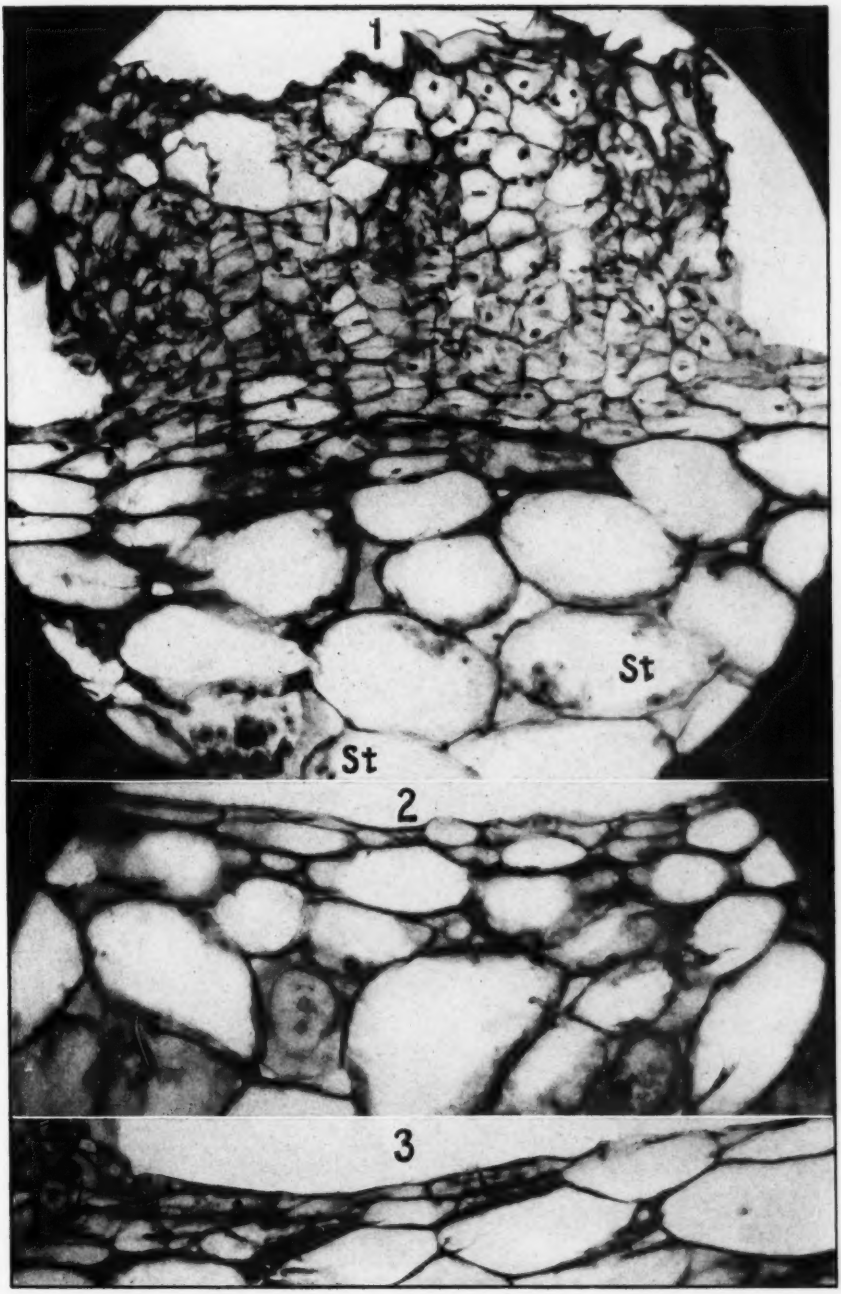
1, 2.—Inner face of carpel wall of green tomato fruit showing proliferations due to ammonium tartrate (20 per cent solution). The growths are from the lining membrane which is normally one cell thick, as shown on the right side of the upper figure, and in Plate 8, figure 2. Injected June 10, 1916. Collected on June 27, 1916. Time, 17 days. ×160. Block 1252 B 5. Cells shriveled more or less by the fixative.

PLATE 8

1.—From the same series of sections as the figures on Plate 7, but showing a more copious proliferation of cells. It is clearly a proliferation from the lining membrane. Carbol-fuchsin stain. Starch (*st*) in the deeper cells. The dark bodies in the tumor cells are nuclei. ×160. Block 1252 B 4.

2.—Normal tissue of inner face of tomato carpel in vicinity of figure 1, for comparison with that and with figures on Plate 7. Same magnification.

3.—Lining membrane of figure 2, passing into such a tumor as figure 1. ×160.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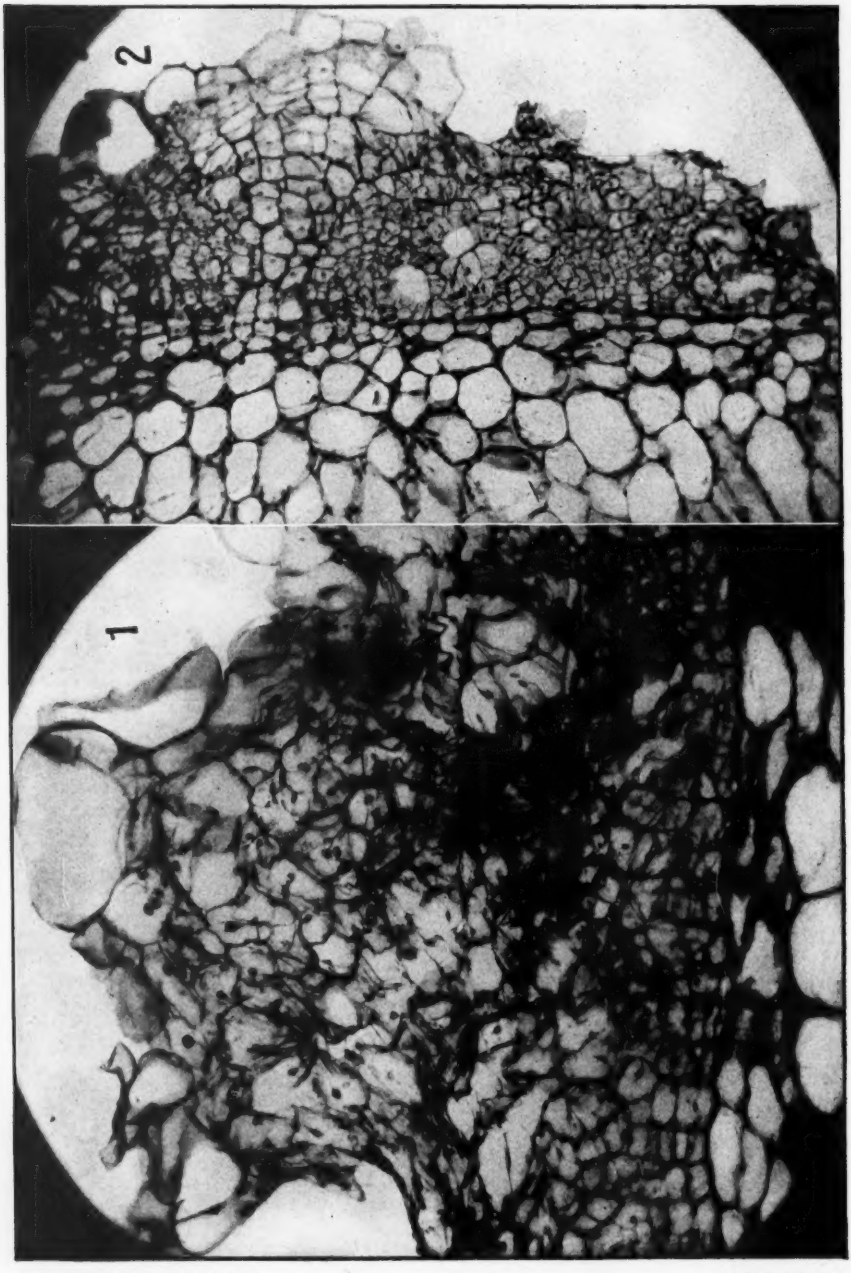
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PLATE 9

1, 2.—Same slide as Plate 7, but showing greater variations in the size of the proliferated cells. Figure 1 was made with the 8 mm. and figure 2 with the 16 mm. lens. The tumors have grown wholly from the lining membrane. Block 1252 B 5. ×160 and 75, respectively.

PLATE 10

Longitudinal section of a stem of *Ricinus communis* injected July 18, 1916, with distilled water containing 10 per cent malic acid. Photographed on July 28. ×9. There is a copious proliferation from the outer pith cells filling the pith cavity. Malic acid in small amounts is a normal constituent of the plant cell.

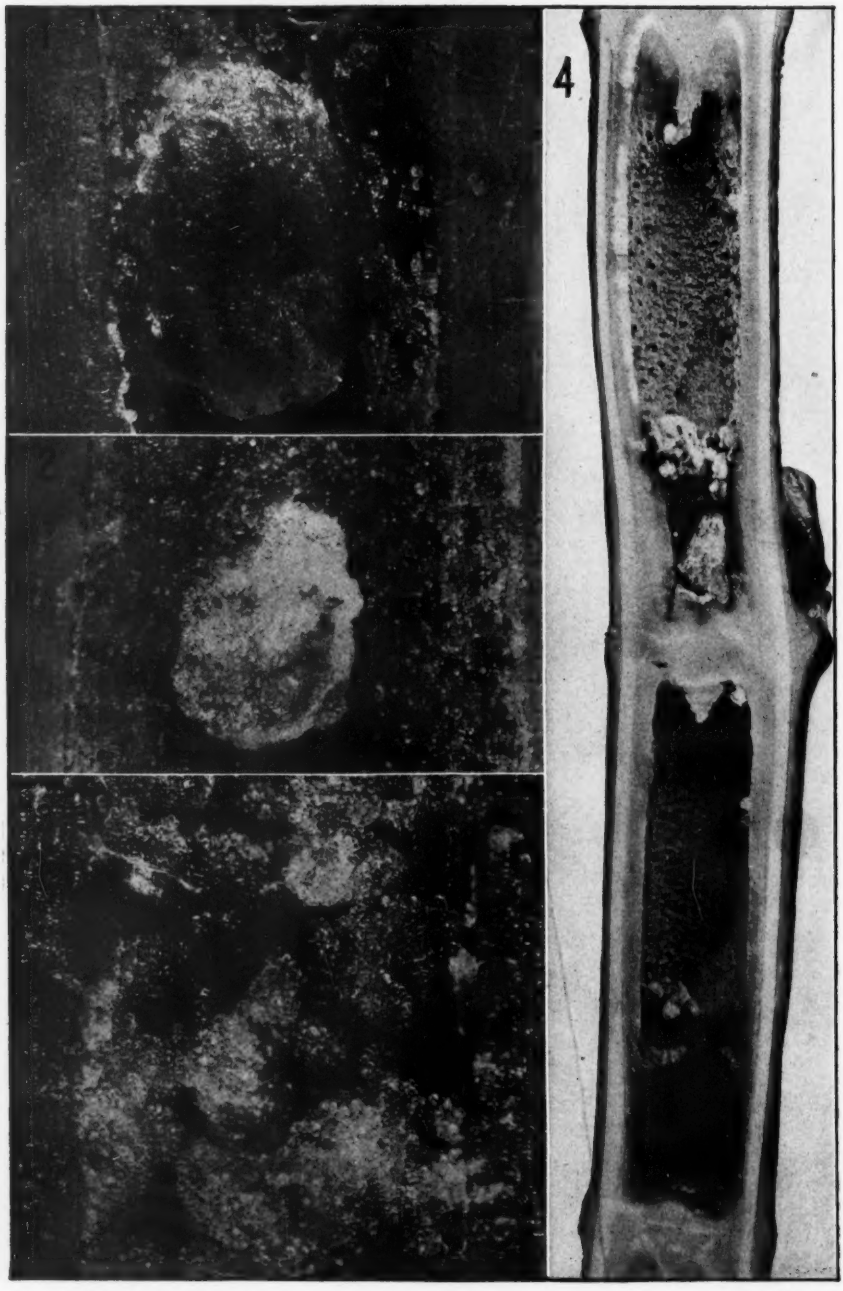
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PLATE 11

1. Middle of an internode of *Ricinus communis* injected July 18, 1916, with 10 per cent malic acid, showing copious surface pith proliferations. The central large nodule developed around the needle entrance. Photographed on July 28, 1916. ×8.

2.—Middle of a Ricinus internode injected July 18 with 10 per cent uric acid. Surface of pith covered with granulations. The central large nodule developed around the needle entrance. Photographed on July 31, 1916. ×12.

3.—Middle of a Ricinus internode, showing copious tumor-like proliferations due to the injection of a 20 per cent solution of urea. These proliferations were yellowish rather than a pure white, as in most cases. Injected June 29, 1916. Photographed on July 11. ×11.

4.—Two Ricinus internodes injected with a 10 per cent solution of tannic acid on August 7, 1916. Photographed on October 20. Natural size. Pith killed and black in the lower third of each; in the upper two-thirds a continuous sheet of proliferations from the pith surface with occasional nodules. The lower large nodules are wound reactions from deeper tissues. Each ceiling also shows proliferations. The killed part of the lower internode was examined for crystals of calcium oxalate but only an occasional crystal was found, not more than would occur in normal stems of this age (see Pl. 46).

PLATE 12

1, 2.—Internodes of *Ricinus communis* injected August 12, 1916, with a 5 per cent water solution of sodium bicarbonate. Cavity filled with copious wound proliferations, all of which are from the pith. The light-colored longitudinal band on either side of the pith cavity shows the extent of the thickening. Killing effects as fissures are visible here and there in figure 1, especially in the inner (sectioned) part of the pith on the right side and more abundantly in figure 2 as caps on the nodules. Photographed on August 25. ×5.

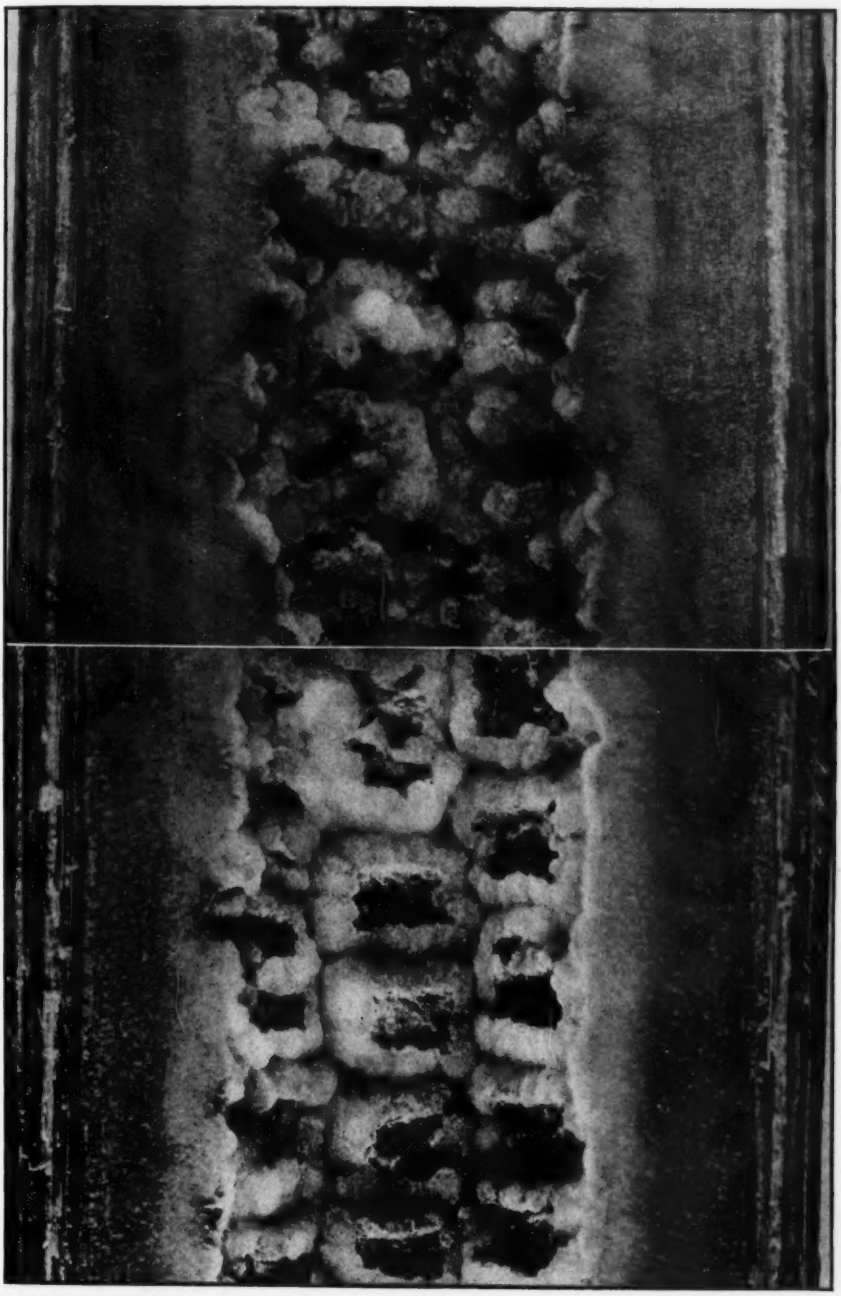

PLATE 13

Internode of *Ricinus communis* showing a pith cavity occupied by numerous anastomosing, white glistening strands and small nodules lying loosely connected on the surface, and due to injection on August 12, 1916, of a 5 per cent solution of ammonium bicarbonate. This is an unopened internode—the second internode above the uppermost injected one. Portions of the injected internodes were killed. For the normal appearance of such an internode see Plate 37, figure 1, and branches of Plate 14, figure 1. Photographed on August 25. ×5.

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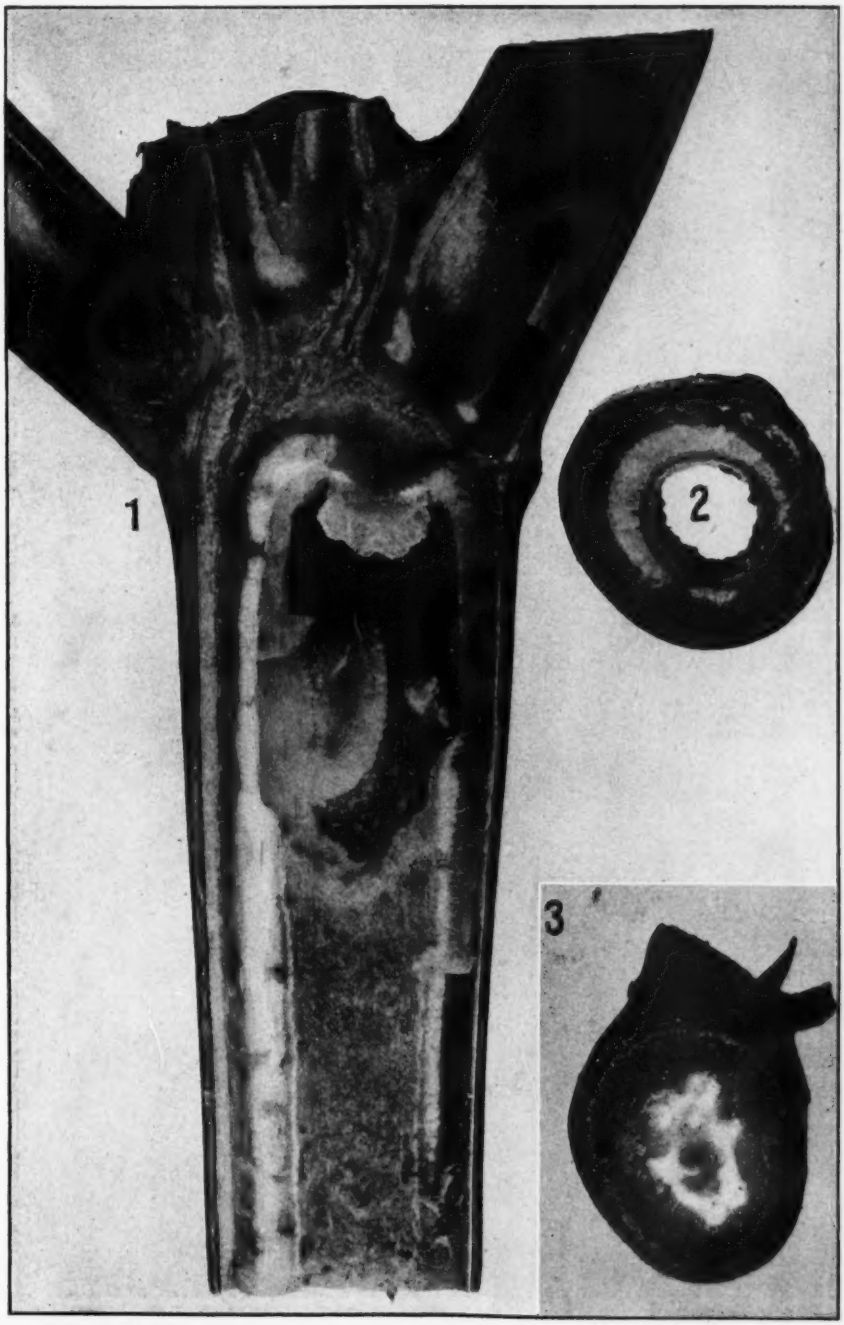
PLATE 14

1.—Longitudinal section of a stem of *Ricinus communis*, showing copious proliferations due to a 5 per cent grape-sugar solution and restricted to the injected internode. Entire wall covered with granulations in the lower part of the figure. The large nodule below the black-paper background developed around the needle entrance. The white band behind this at the left and on the lower right side is dead pith. ×2.

2.—Cross section below figure 1. The dead pith (white ring) encircles the inner, still living granulations. Injected August 7, 1916. Photographed on August 24. ×2.

3.—Cross section of a Ricinus internode which received 0.2 c. c. of clear lime water when young. No evidence of wound-repair tissue, but the pith cavity filled solid (save for a black speck in the center) with pith cells as in *a very young* internode. The white area represents the line along which the outer pith would later have separated from the central core. There was much diffuse red stain in the living pith outside of the white (dead) area, and this pigment which was also abundant in the internodes injected with sodium bicarbonate stained Carnoy's fluid decidedly red. This red stain is perhaps to be accounted for by an excessive movement of sugar into the treated internodes. The normal diameter of the pith cavity of a Ricinus stem of this age and shape would be about 17 by 12 mm. Internode injected on August 8, 1916. Photographed on August 24 about 1 inch above the lower node. Only the lower 2.5 cm. of the lumen (part below the needle entrance) was completely filled with tissue. ×1½ (nearly).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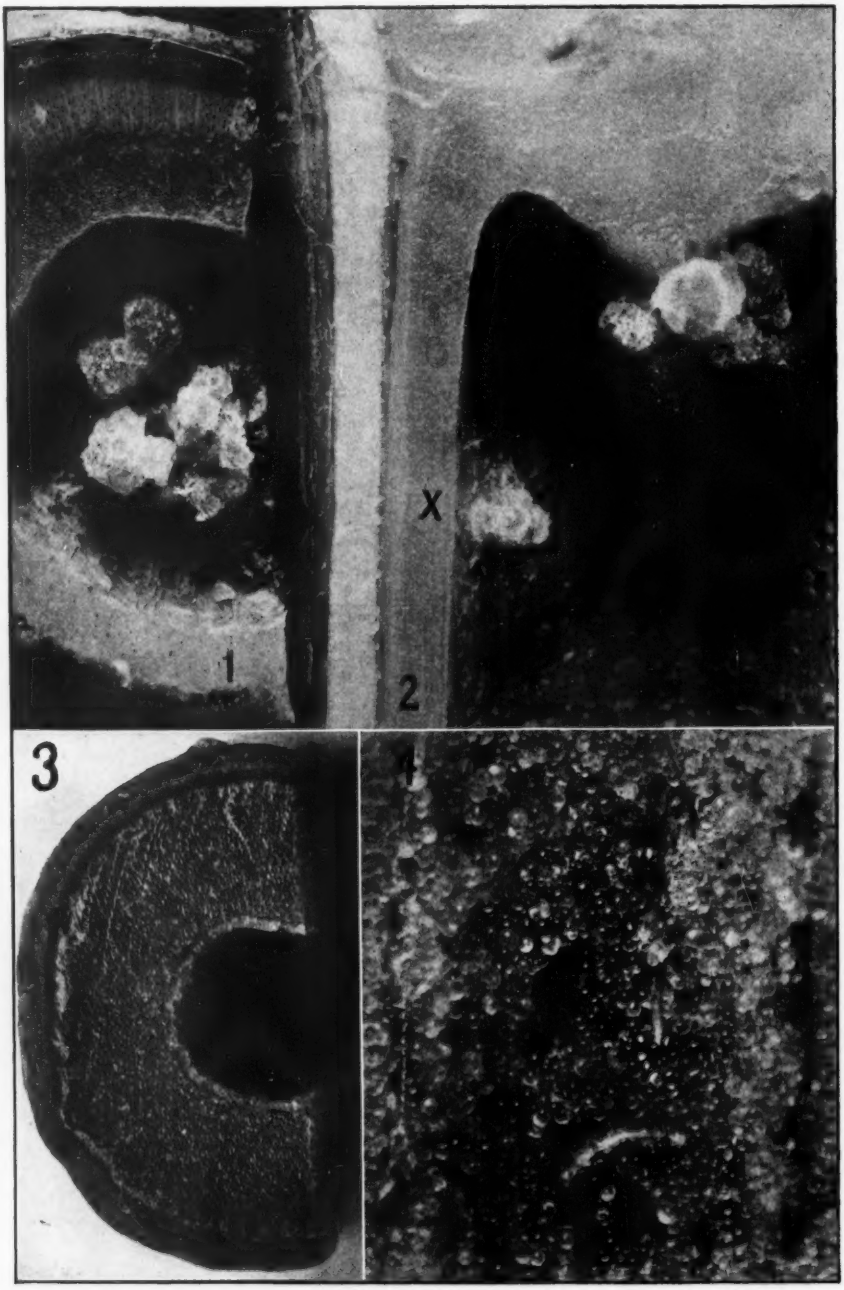
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X
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PLATE 15

1, 2.—Internodes of *Ricinus communis*, showing the result of injecting a 5 per cent solution of cane sugar into young internodes of the main axis, the stimulus being confined to the injected internodes. (1) Ceiling of lower injected internode and (2) ceiling and upper part of middle injected internode, both showing conspicuous proliferations—that is, numerous small tumors. That at *X* is where the needle entered. There is no visible killing of the pith surface in the upper part of either internode. Injected on August 7, 1916. Photographed on October 19, 1916. ×3.

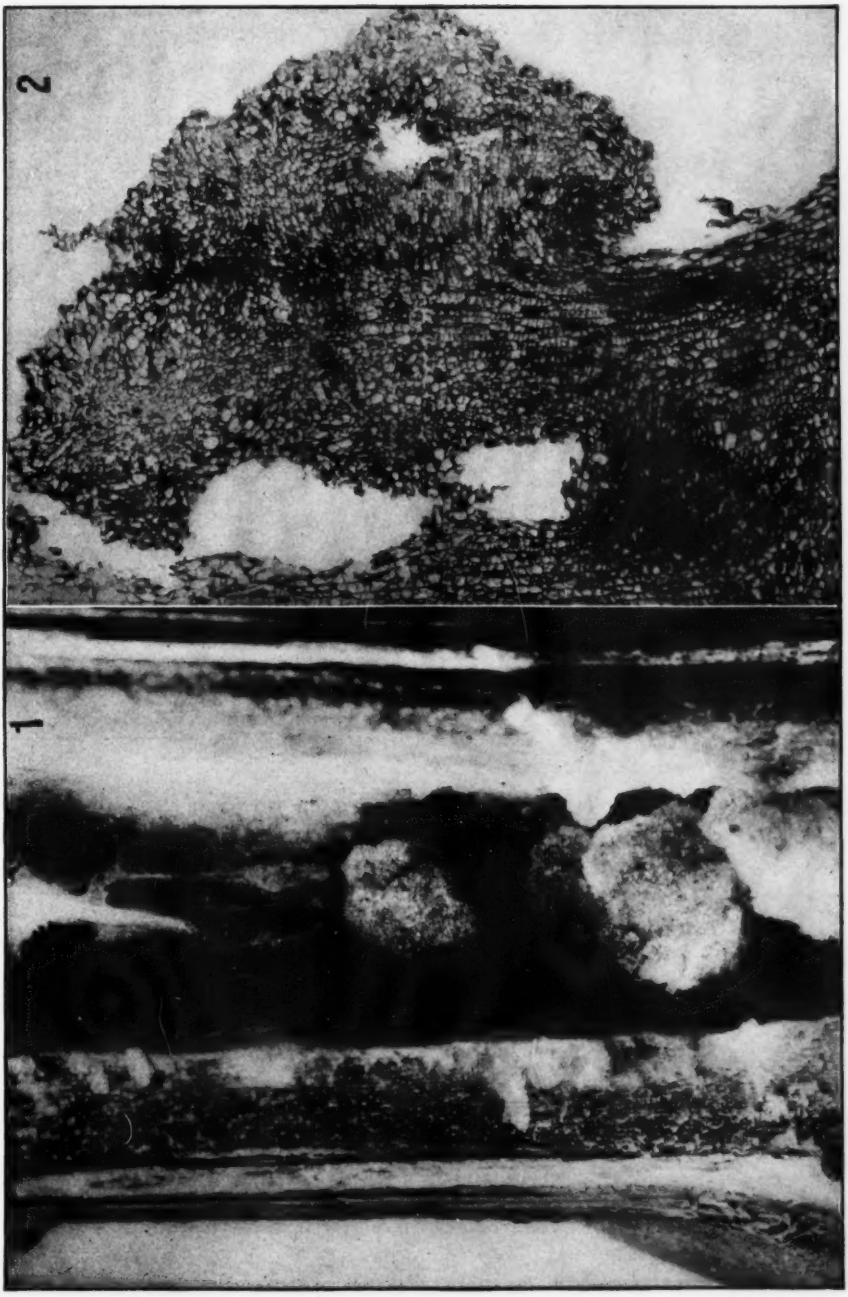
3.—Cross section of upper part of a young Ricinus stem showing pith cavity lined by a thin sheet of glistening white cell proliferations due to the injection of ammonium tartrate. Time, nine days. ×10 (circa).

4.—Side view of figure 3, further enlarged to show the cells.

PLATE 16

1.—Eight small tumors and various strands in an unopened internode of *Ricinus communis*, which showed no surface injury of the pith. On the opposite side of the pith cavity facing these nodules was a continuous white tumor an inch long. This is the first internode above one into which was inserted a small sterile glass tube holding 0.2 c. c. of a 5 per cent solution of monobasic ammonium phosphate, which slowly gives off alkaline vapors, and between the two internodes there was ¾ inch of solid node tissue. Tube inserted on August 8, 1916. Photographed on August 30. ×5.

2.—Section of one of the tumors shown in figure 1. Four unopened internodes in this stem showed effect of the vapor—namely, the first one below that which held the tube and the three immediately above it.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1
2

PLATE 17

1, 2.—Cross sections of two internodes of *Ricinus communis* (two plants), showing pith proliferations due to the feeble alkaline vapors from monobasic ammonium phosphate. An open glass tube containing 0.2 c. c. of a 5 per cent water solution of the phosphate was inserted into the lower end of these internodes and sealed in on August 7, 1916. The plate shows sections of the upper end of each internode. The lower was more responsive than the upper, probably because younger, although the upper shows granulations from all parts of the pith surface. Photographed on August 17. ×8.

PLATE 18

1.—Top of lower injected internode of plant 1 of *Ricinus communis*, which on August 15, 1916, received 1 to 100 ammonia water (one part aqua ammonia sp. gr. 0.90; 100 parts distilled water) and which shows numerous proliferations from the ceiling.

2.—Section 1 inch below figure 1, but above the needle entrance. This shows proliferations from the side wall with no dark specks on the pith. Photographed on October 3, 1916. ×5.

2
1

2
1

PLATE 19

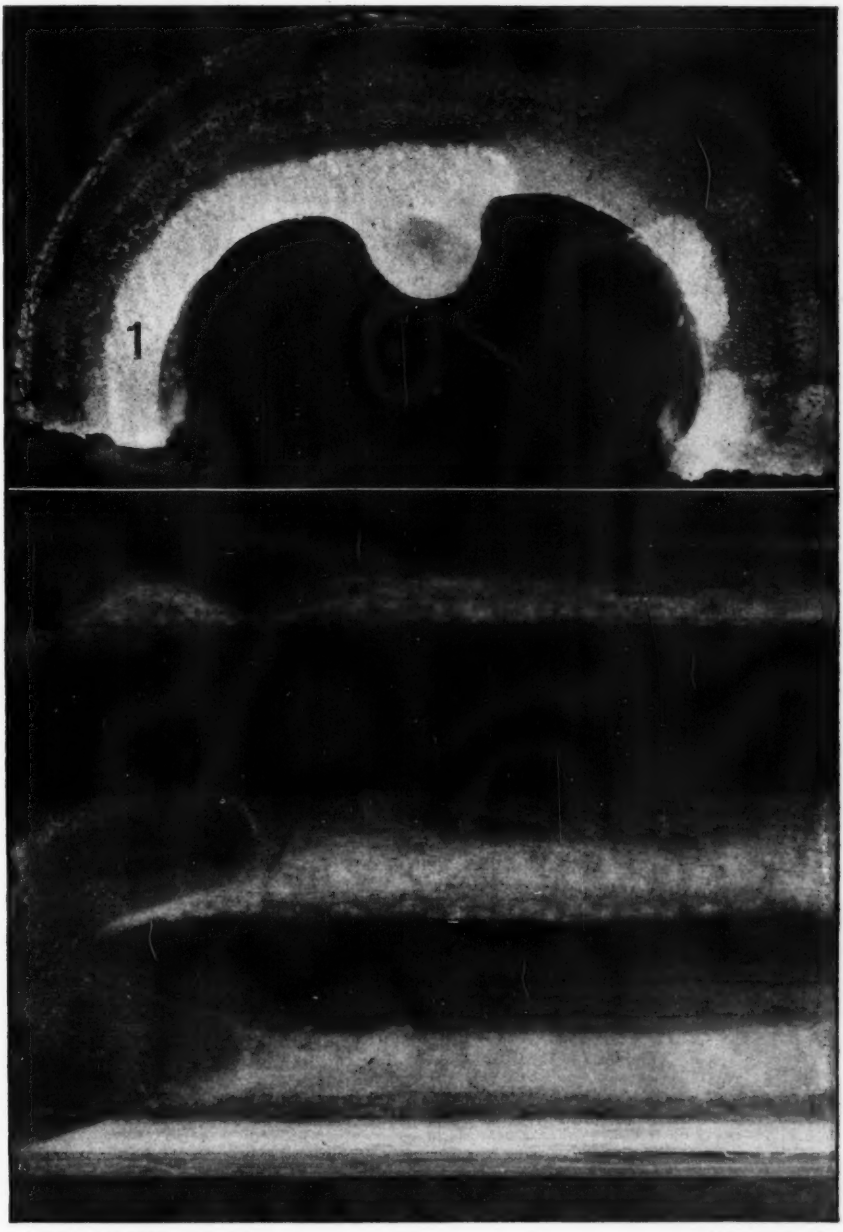
1.—Floor of upper injected internode of the main axis of plant 1 of *Ricinus communis*, showing copious proliferations from the pith. This was injected with 1 to 100 ammonia water on August 15. Photographed on October 3, 1916. ×4.

2.—Same as figure 1, but with the stem pared down one-fourth inch to remove shadows and show the proliferations in their proper color (pure white). Photographed on October 3, 1916. ×4.

PLATE 20

1.—Plant 1 of *Ricinus communis*, which received 1 to 100 ammonia water. Cross section of first (untreated) internode above the one figured on Plate 19, showing on one side an excess of pith, in the middle of which are displaced vascular bundles. There are no surface granulations. ×5.

2.—Side view of the same. The ridge extended the whole length of the 6-inch internode, through the floor of which there was an abnormal opening into the treated internode below. Vascular strands at *X*. Injected August 15, 1916. Photographed on October 3. ×5. For the structure of the strands see Plate 43.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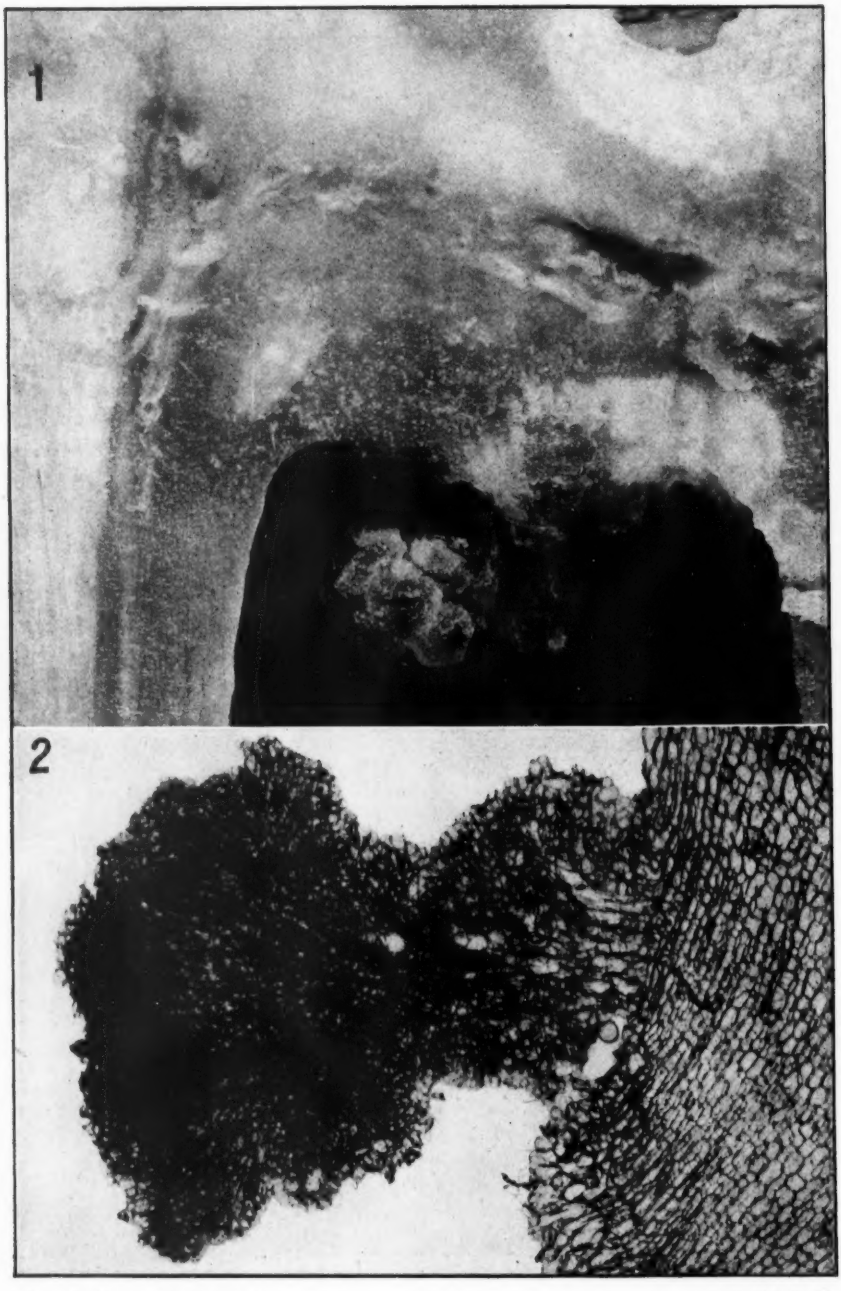
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PLATE 21

1.—Roof of second unopened internode below lowest injected one on main axis of plant 1 of *Ricinus communis*, which was injected with 1 to 100 ammonia water on August 15. This node gave rise to a branch the lowest internode of which had been injected. At the extreme top is shown the floor of the first unopened internode below the lowest injected one on the main axis, and here there are no proliferations, but there was a growth on the ceiling above this floor. Photographed on October 3, 1916. ×5.

2.—Free-hand unstained section of tumor shown in figure 1. The section is too thick; it remained overnight in water, and it is not sufficiently magnified. The right-hand part is normal tissue. Planar f. 35 mm., bellows 55.

PLATE 22

1, 2.—Plant 5 of *Ricinus communis*, injected August 15, 1916, with 1 to 1,200 ammonia water (aqua ammonia sp. gr. 0.90, 1 part, distilled water 1,200 parts). Both faces of the upper part of the second injected internode (main axis) show knobs and glistening strands of proliferated pith tissue. Below there was a continuous sheet of glistening proliferated cells. There were evidences of pith injury in the lower pat of the internode but none in this part. Photographed on September 11. ×3.

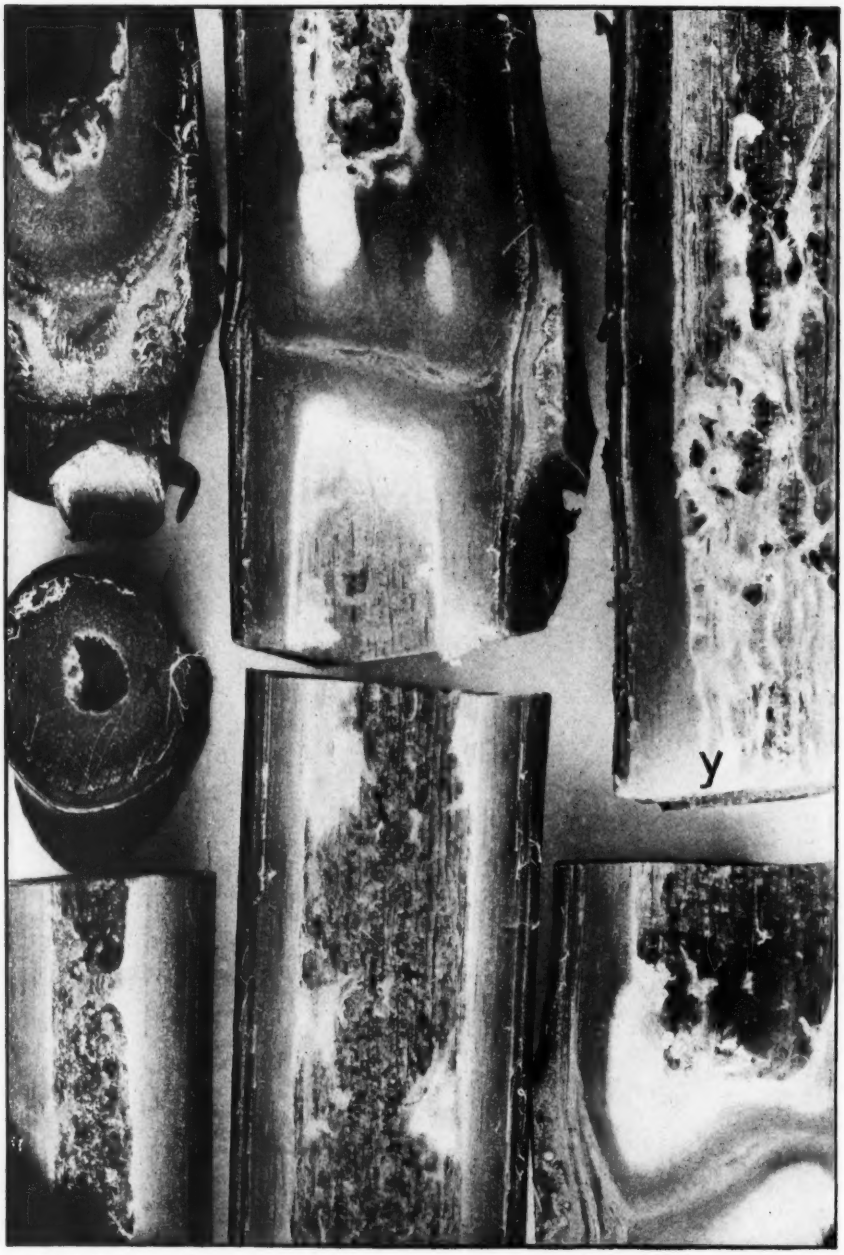
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PLATE 23

Plant 5 of *Ricinus communis*, branch A, showing effect of 1 to 1,200 ammonia water. These are sections of the fourth (X), third, second, and first internodes of the uppermost untreated branch.

All show white nodular proliferations, and Y also shows strands; none were injected; and none show pith injury (dark specking). This branch was very young or not yet developed at the time of the inoculation, but it grew out of that node of the main axis which formed the ceiling of the uppermost injected internode (see Pl. 27, fig. 3). Somewhat below the surface, in the shadow, on the right side of the pith wall of X, are also half a dozen small tumors. Plant injected August 15, 1916. Photographed on September 11. ×3.

PLATE 24

1.—Plant 5 of *Ricinus communis*, branch B, injected August 15, 1916, with 1 to 1,200 ammonia water, showing proliferations from the ceiling and side walls of the second internode above the uppermost injected one. There are half a dozen side-wall tumors, as well as the central one. Surface of the pith uninjured. ×5.

2.—Section of small marginal proliferation on wall of pith cavity shown in figure 1. ×160. Compare with Plate 29, figure 6.

3.—Same as figure 1, but from branch C. This is the third internode above the uppermost injected one. There were also proliferations in the first and second internodes above the injected one. Here the proliferations are all on one side in a perpendicular line, all are inconspicuous elevations, and the pith surface as a whole is entirely normal. Photographed on September 11, 1916. ×3.

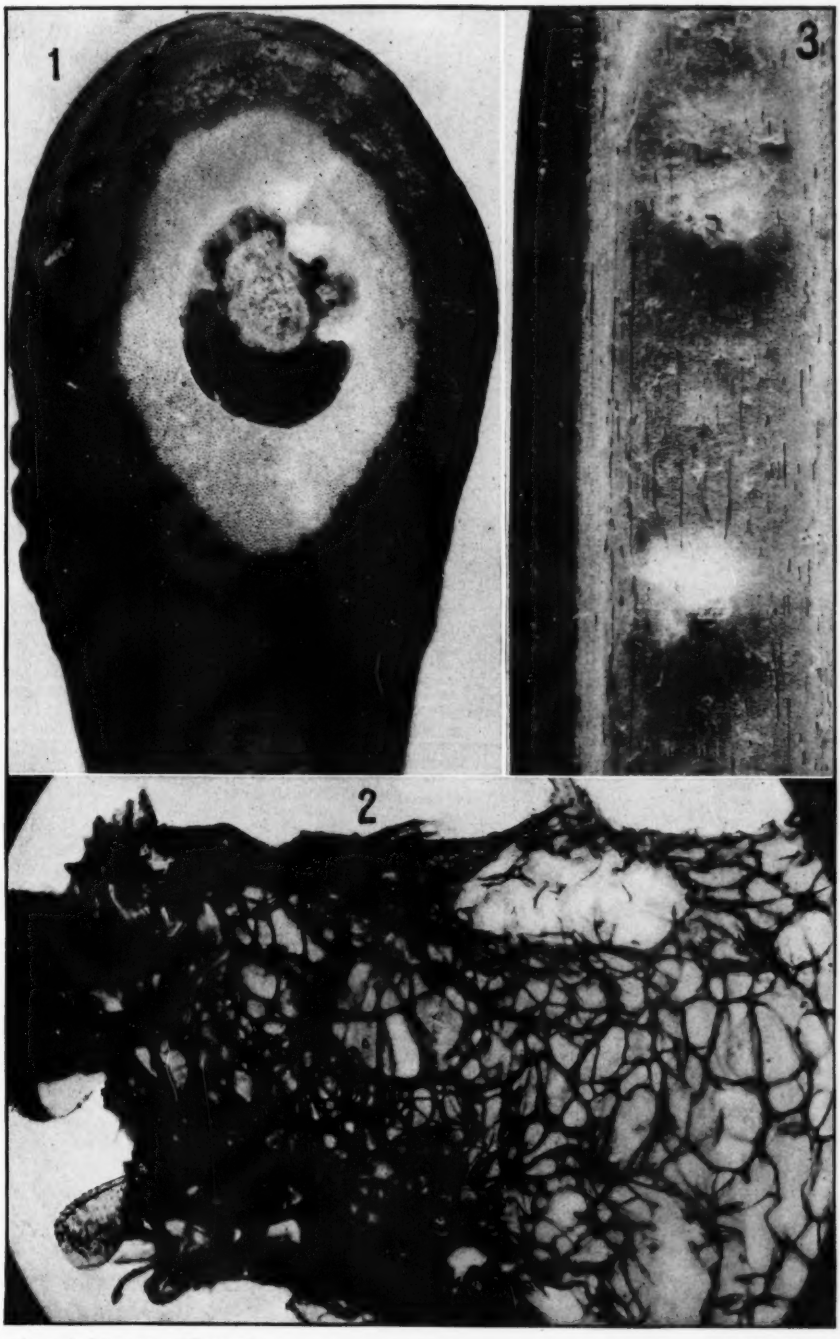
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PLATE 25

Upper half of first internode above the upper injected one in the main axis of plant 5 of *Ricinus communis*, which was injected August 15, 1916, with 1 to 1,200 ammonia water. The wall is thickened and there are numerous small proliferations—stringy growths and nodules. The surface pith between is normal, but the deeper layers of the pith are white (occupied by air). Photographed on September 11. ×5.

PLATE 26

Lower half of same internode as Plate 25, showing continuation of tumors and strands. ×5.

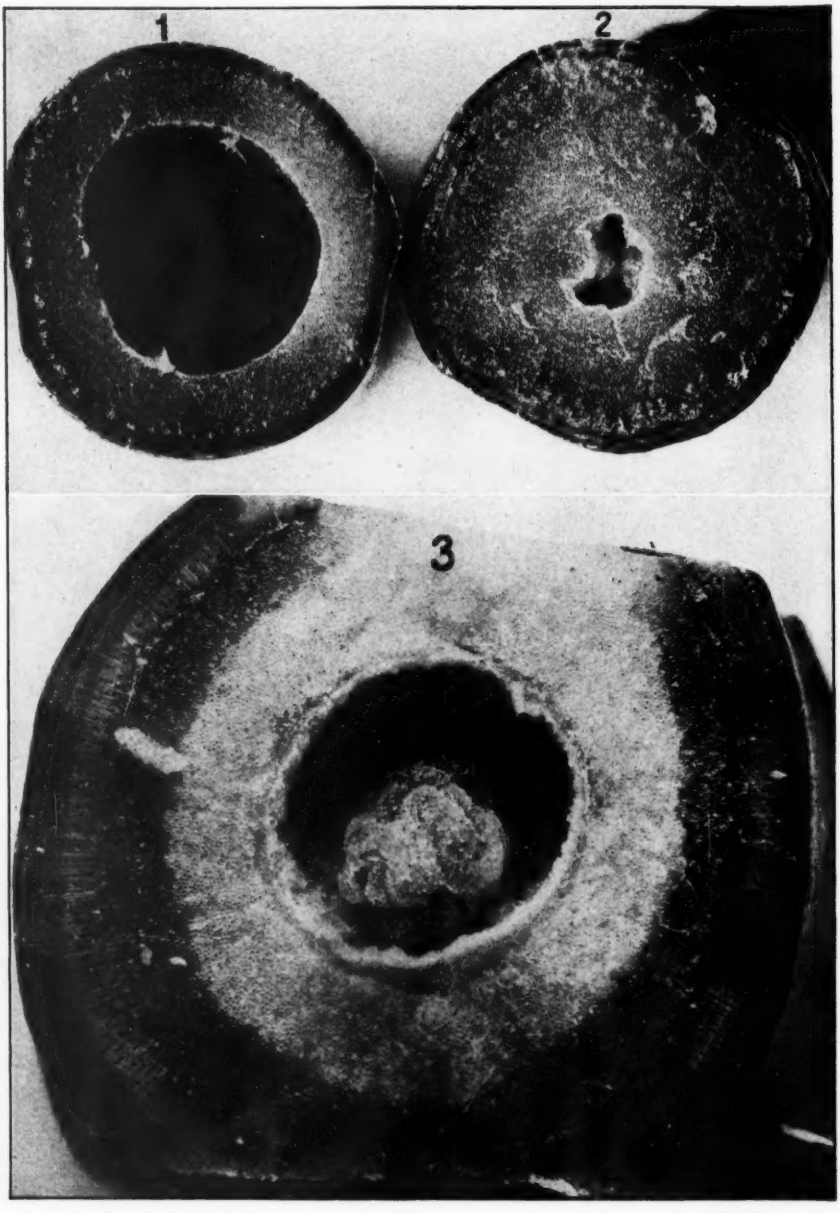
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PLATE 27

1.—Cross section of a normal internode of the same age as figure 2.

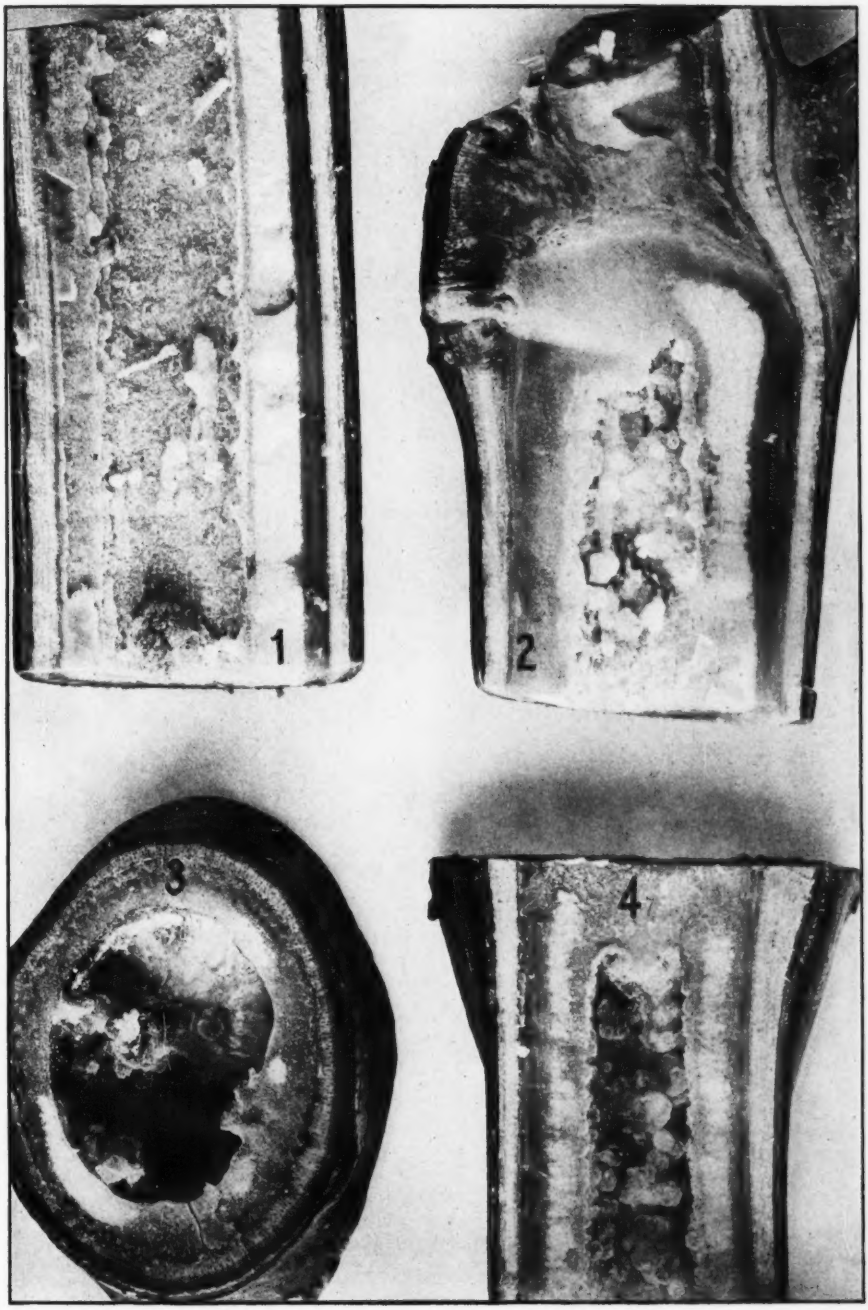
2.—Main axis of plant 5 of *Ricinus communis*, injected August 15, 1916, with 1 to 1,200 ammonia water. Cross section of fourth internode above the uppermost injected one showing a much thickened wall and nodular proliferations from opposite sides of the inner pith, which did not show any dead cells. Every one of the four internodes above the uppermost injected one showed striking proliferations from the side walls and floors and no specking of the pith—that is, no surface injury. Photographed on September 11. ×4.

3.—Main axis of Ricinus plant 5, injected with 1-to-1,200 ammonia water, showing nodular proliferations from the ceiling and thickened sides of the uppermost injected internode. There was also a sheet of proliferation 1 mm. thick over the whole inner surface of the internode, and on this there were about 50 protuberant small tumors (glistening white cell masses). There was no dark specking of the pith, but the deeper layers were white—that is, permeated by air. The floor also bore nodules like the ceiling. Photographed on September 11. ×5.

PLATE 28

1, 2, 3, 4.—Plant 4 of *Ricinus communis*, injected August 15, 1916, with 1 to 1,200 ammonia water. Various portions of injected internodes all the proliferations of which are from the inner pith. In figure 1 there is a continuous sheet of granulations bearing nodules and strands.

Figure 3 is a ceiling. Photographed on September 11, 1916. ×2.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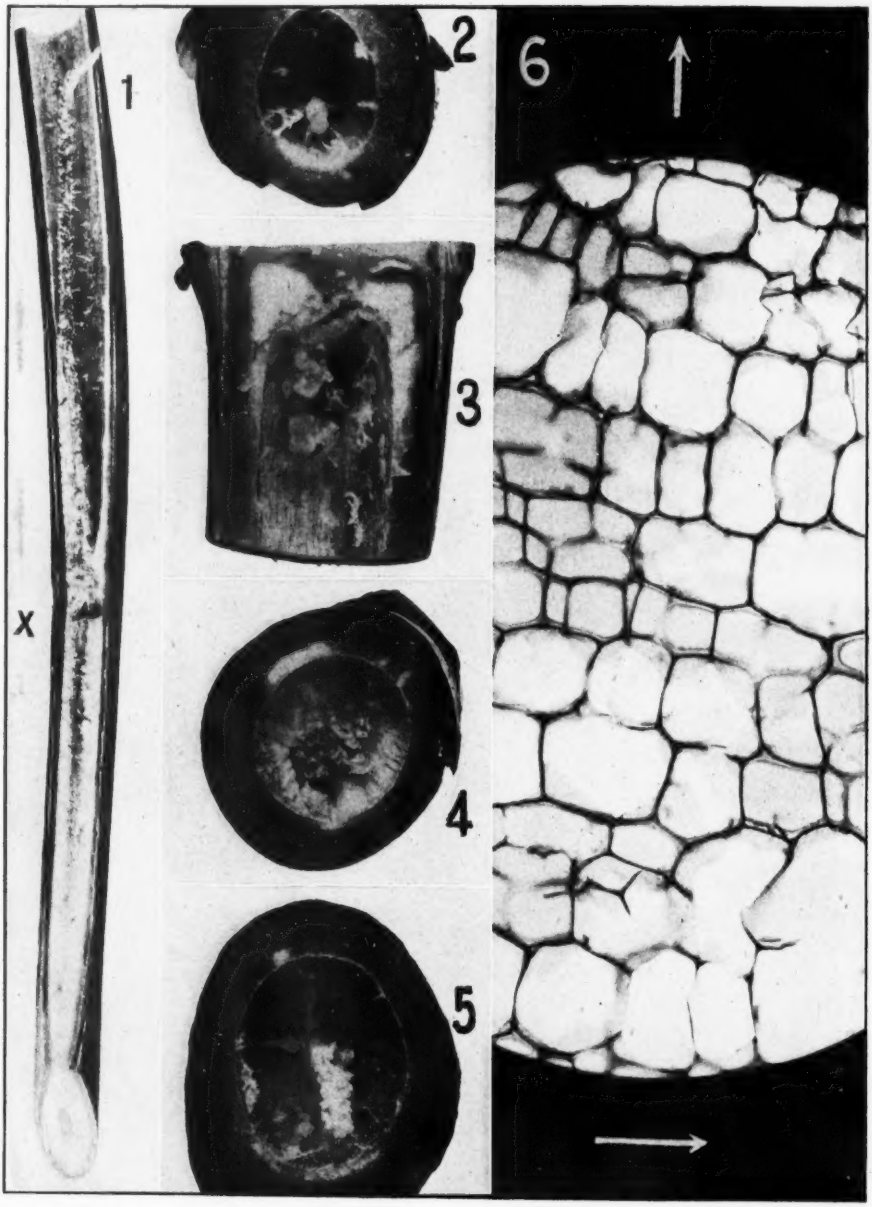
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6
X

PLATE 29

1–5.—Petiole and stem sections of plant 4 of *Ricinus communis*, which was injected on August 15 with 1-to-1,200 ammonia water.

1.—Middle of petiole, needle entrance at X. Pith much thickened (proliferated) and torn away in the upper part, leaving a rough-coated hollow cylinder of proliferated tissue. (See also Pl. 37 and 42.)

2, 3, 4, 5.—Parts of four unopened internodes at the top of the plant—that is, remote from the injected internodes, showing white glistening nodules in tissues which developed mostly after the injection. One of these sections is from a branch and the others are from the main axis. Between the uppermost injected internode and these nodules two and three nodes intervened. Photographed on September 11, 1916. Petiole natural size. Figures 2 to 5, × 2.

6.—Normal pith from Plate 24, figure 1. It joins on (in direction of the upper arrow) to right side of Plate 24, figure 2. Arrow at bottom indicates the longer axis of the stem. ×160.

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PLATE 30

Ceiling of upper treated internode of plant 2 of *Ricinus communis*, which was injected with 1-to-1,200 ammonia water on August 15, 1916. It shows lobed tumors from the center and also from a dozen places on the side walls. There was here no trace of killing action (surface specking) of the pith. Photographed on September 9, 1916. ×5.

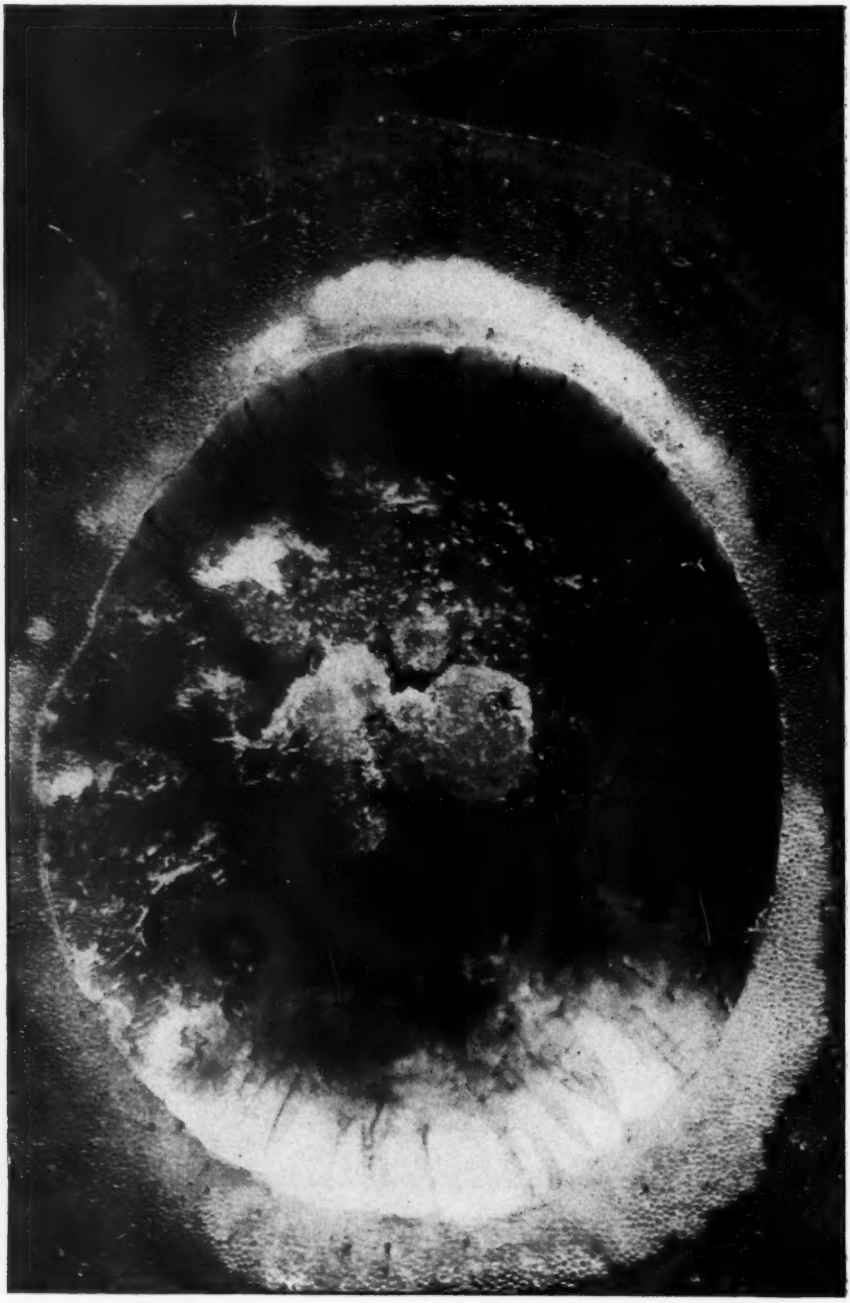
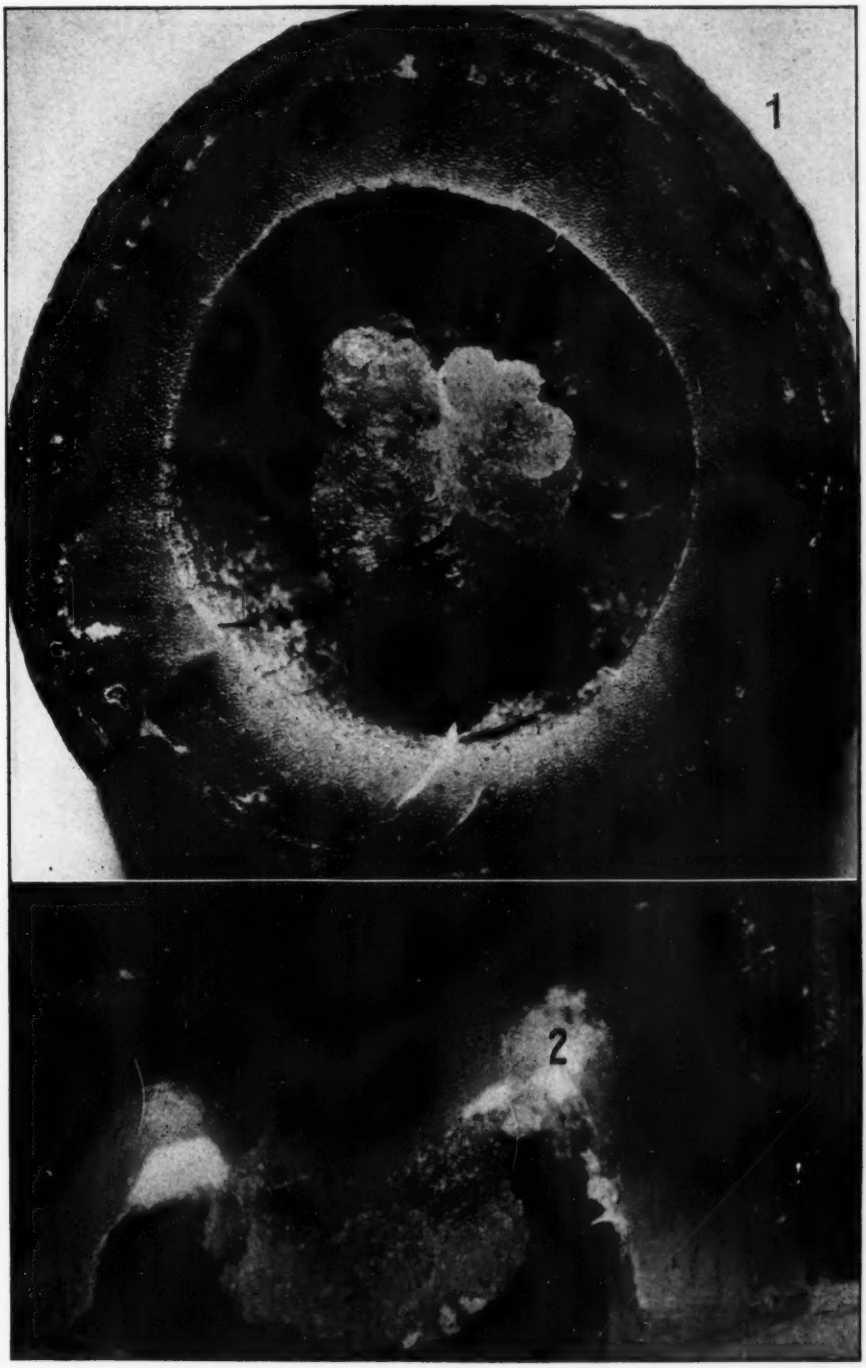


PLATE 31

Ceiling of second (middle) injected internode of plant 3 of *Ricinus communis*, which received 1-to-1,200 ammonia water on August 15, 1916. It shows granulations and nodules and no killing of the tissues. The dark marginal stripes on the cavity wall are normal red stripes due to superposed pigmented pith cells which also occur freely in the deeper parts of the normal pith of *Ricinus communis*. Photographed on September 9. ×5.

PLATE 32

1, 2.—Stem of *Ricinus communis* exposed to the vapors of monobasic ammonium phosphate (20 per cent in water). Figure 1 is a front view and figure 2 a side view of nodular growths which developed on the ceiling of the unopened internode next below the one into which was sealed a sterile glass tube containing 0.2 c. c. of the fluid. Experiment begun August 21, 1916. Photographed on September 2. ×5.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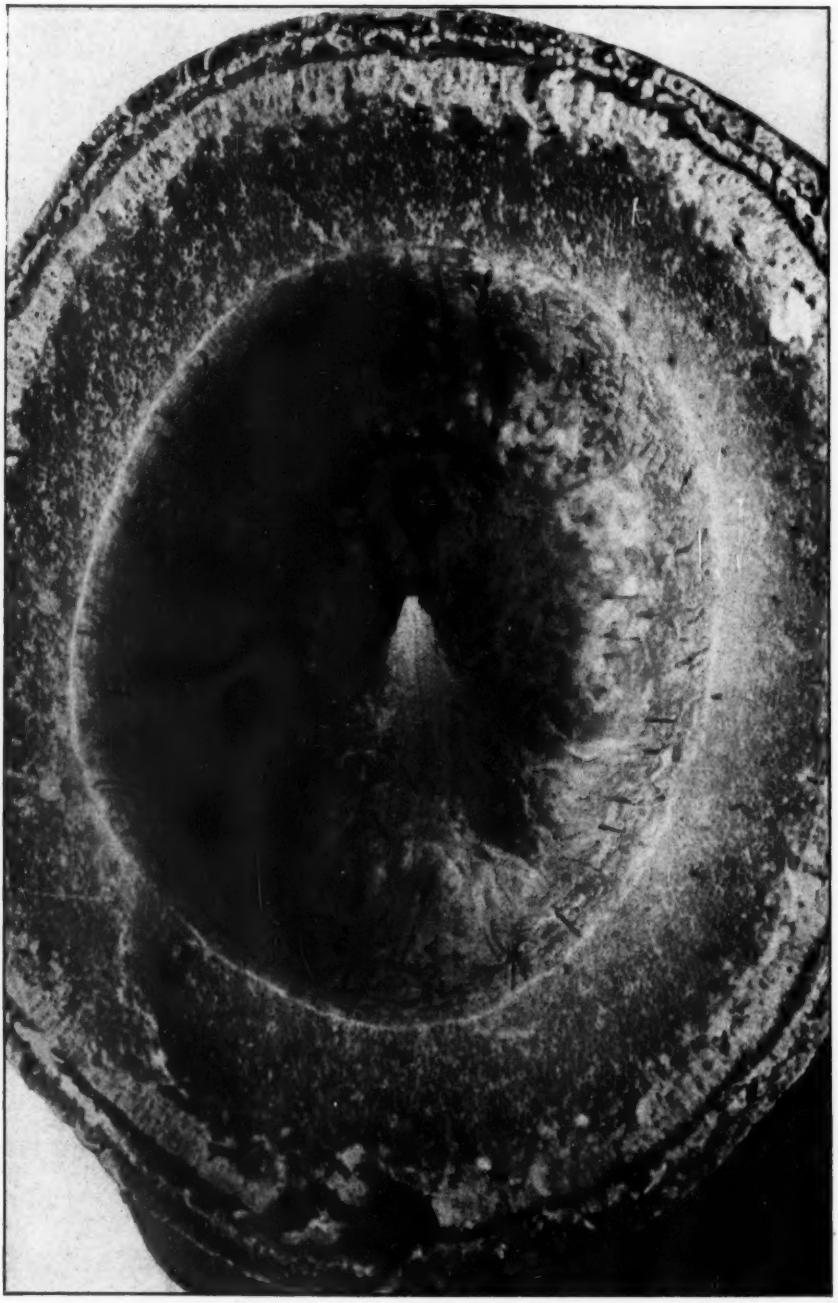

PLATE 33

Stem of *Ricinus communis* in cross-section showing pith-lining of a normal node for comparison with Plates 32, 34, and others. The middle of the node wall often bears a smooth, cone-shaped projection as at *X*. Photographed on September 5, 1916. ×8.

PLATE 34

Longitudinal section of stem of *Ricinus communis* showing further effects of vapor of monobasic ammonium phosphate. Same plant as shown on Plate 32, but in another place—that is, in the upper end of the tube internode showing ceiling and side-wall tumors, and the lower end of the unopened internode above bearing a floor tumor, the tip of which (*X*) is normal (see Pl. 33). Exposure begun on August 21, 1916. Photographed on September 2. ×5. For a longitudinal section through a normal node of the same age see Plate 35.

X

VB
VB
VB
Xy

PLATE 35

Longitudinal section through a normal node and part of the two adjoining internodes of a well-developed plant of *Ricinus communis*. *B*, Bark; *Xy*, wood; *P*, pith. Bundles of anastomosing vessels (*VB*) are visible in the middle of the node as white bands. ×5.

PLATE 36

Top of an unopened internode in a stem of *Ricinus communis* next under one that had sealed into it a tube containing 0. 2 c. c. of a 20 per cent solution of monobasic ammonium phosphate. It shows a thickened cone and nodular proliferations from its apex. The white patches in the pith are dead cells and below the surface here shown they are united. In cross section at the extreme left are the vascular bundles of the node showing as dark spots (*VB*) and above and below as strands. Experiment begun August 21, 1916. Photographed on September 5. ×5.

VB

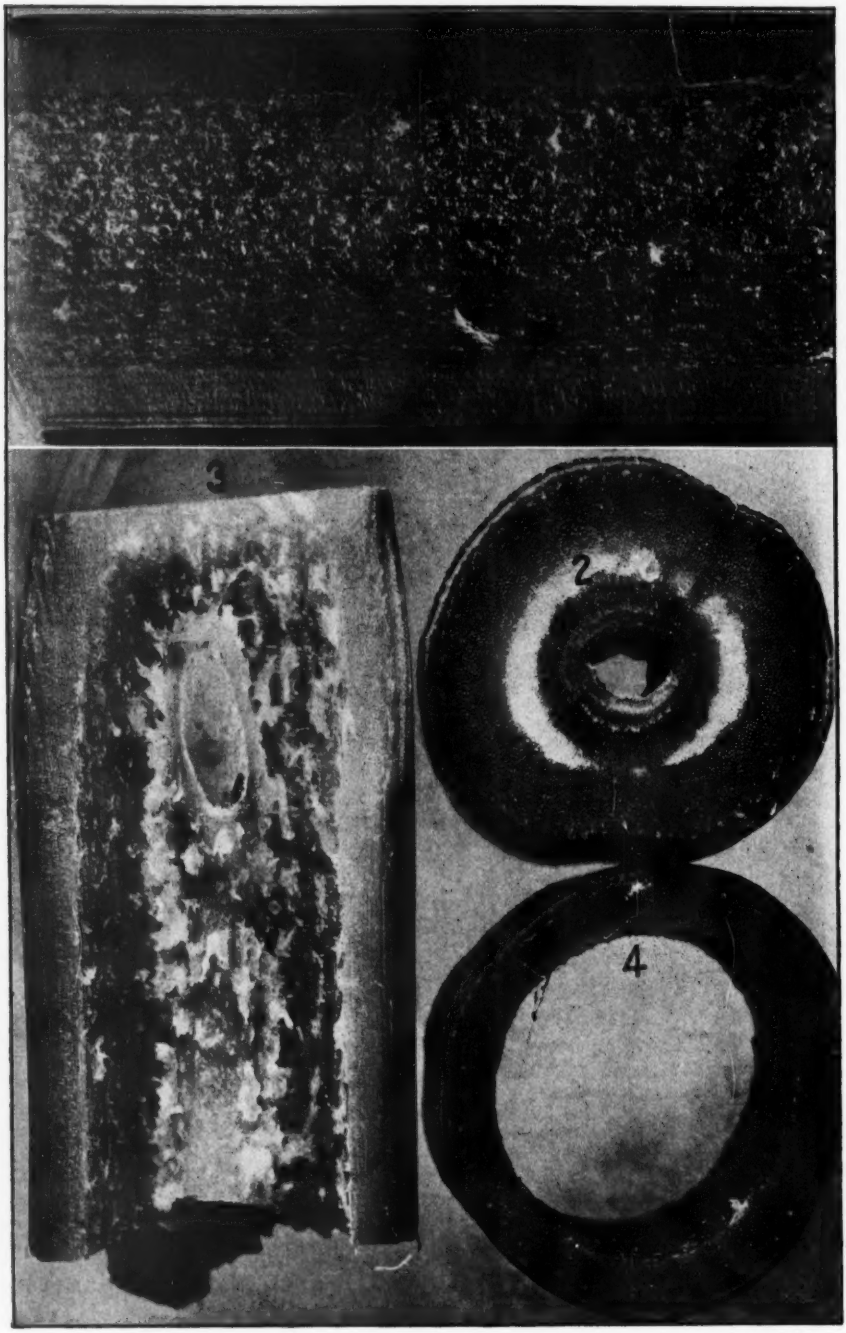
3
2
4

PLATE 37

1.—Longitudinal section of a normal young internode of *Ricinus communis*. The white specks are shriveled (dead) pith fragments dating from the formation of the pith cavity. Photographed on July 27, 1916. ×4.

2.—Cross section of a Ricinus internode into which on July 22 was injected a 5 per cent solution of monobasic ammonium phosphate. This caused a great thickening of the pith with a continuous sheet of surface granulations behind which a second xylem-phloem cylinder has developed and between which and the normal xylem-phloem cylinder is a wide area of pith, the middle part of which is losing water (becoming white) and preparing to form a cylindrical cavity. At other levels this cavity was already visible. Time 12 days. ×4.

3.—Injected internode of another plant in the same series. Here the proliferated pith, the inner part of which is still living, has split away entirely from the outer normal pith and lies as a rough-coated hollow cylinder loose in the pith cavity. The white flecks on its surface are torn masses of dead and shriveled pith cells. ×4.

4.—Cross section of a normal internode of the same age as that in figure 2, for comparison with the latter. Photographed on August 3, 1916. ×4.

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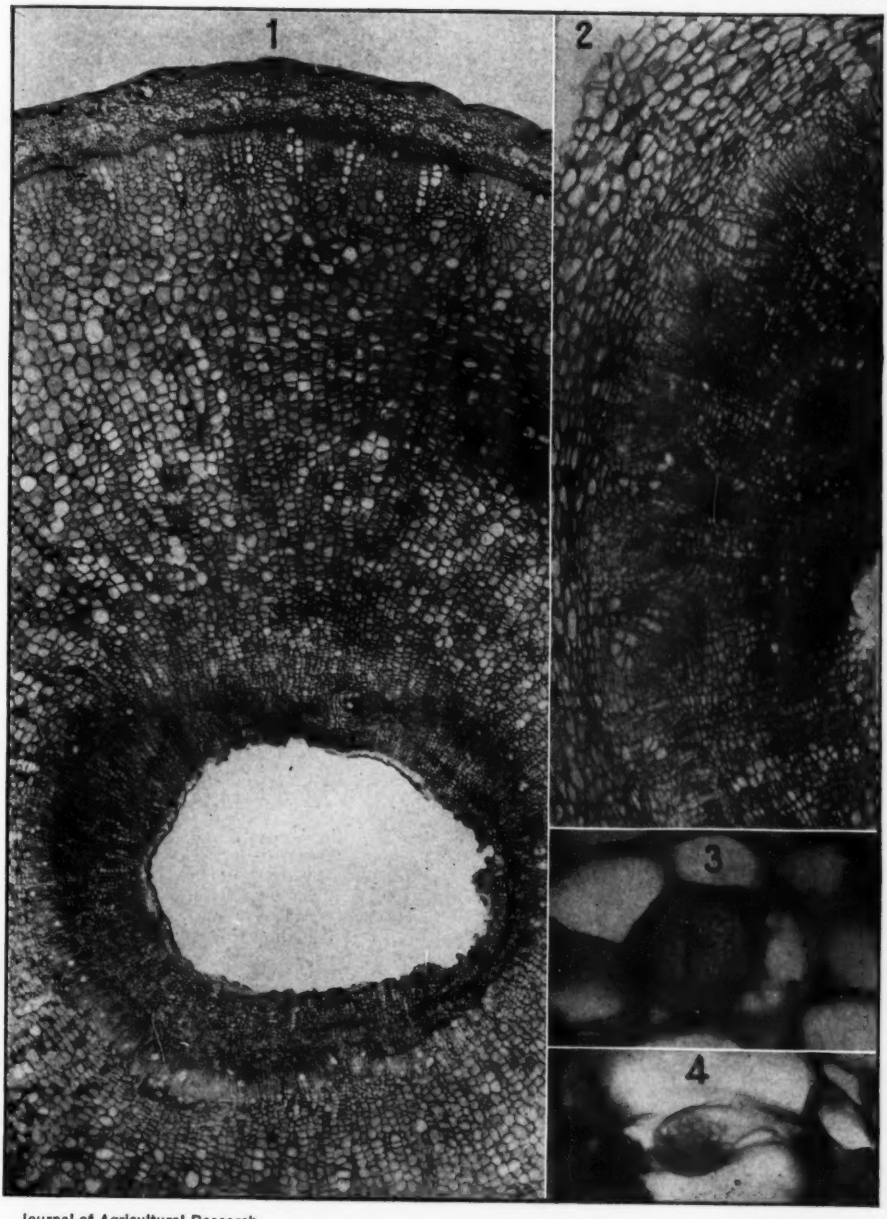
PLATE 38

1.—Same as Plate 37, figure 2, but from an embedded stained section. It can be seen that the pith cavity is irregular and that the second xylem-phloem ring is farther from the pith cavity on the left side than it is on the right side. It is also more irregular than the normal xylem phloem—that is, at top and bottom. The heavily stained cells in the pith are those which show white (dead) in Plate 37, figure 2. The interrupted dark line a little way in from the pith cavity is another cambium, not accompanied by vessels.

2.—A detail of the second xylem-phloem ring showing irregularities more distinctly. Planar enlargement.

3.—Sieve plate from second xylem-phloem ring. ×650.

4.—Distorted sieve tube from third (imperfect) xylem-phloem ring. ×650.


1
2
3
4

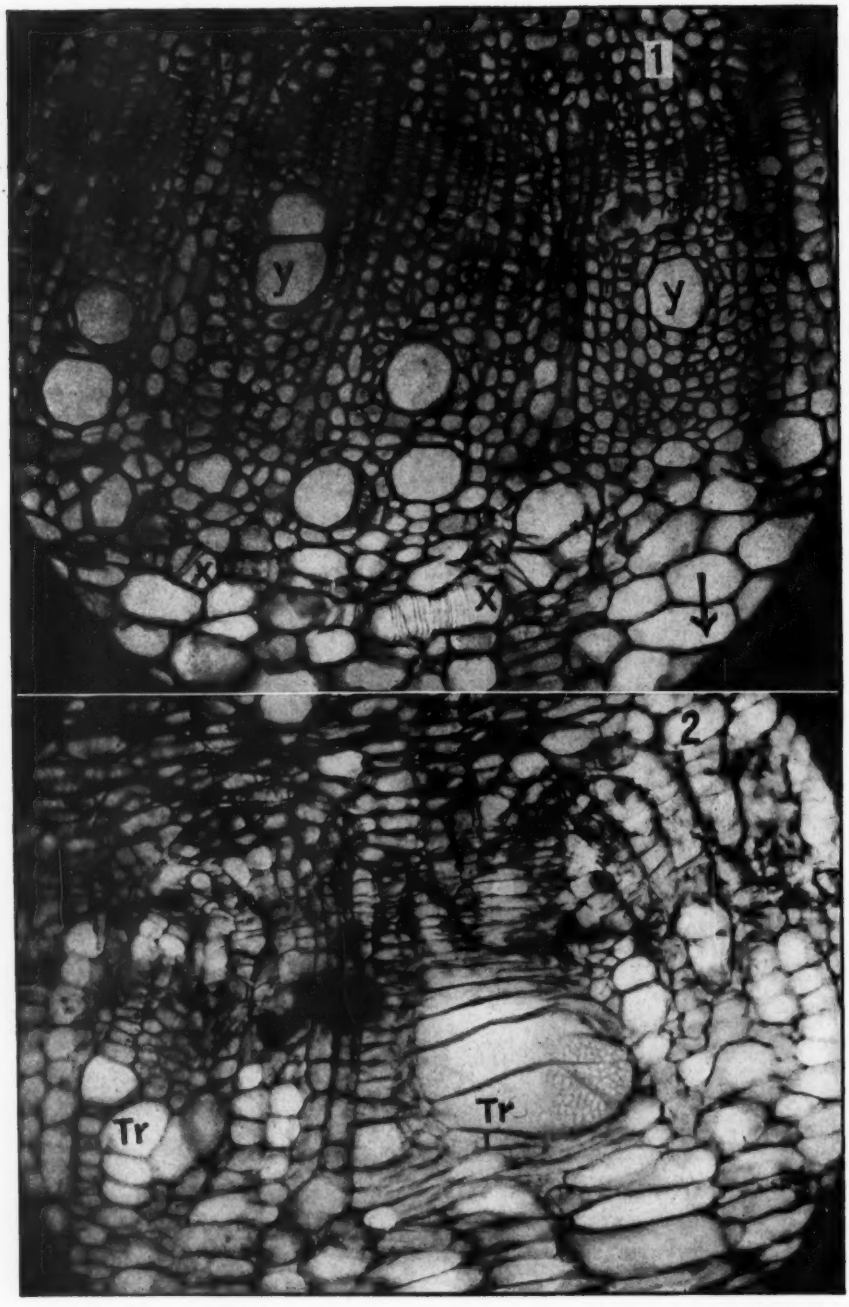

1
y
y
x
x
2
Tr
Tr

PLATE 39

1.—Enlarged cross section of a rather regular portion of the second xylem-phloem ring in *Ricinus communis* (Pl. 37, fig. 2). The normal xylem-phloem ring is in the direction of the arrow. Cambium and phloem at the top. Twisted spiral vessels at *X*. Tracheæ properly oriented at *Y*. ×160.

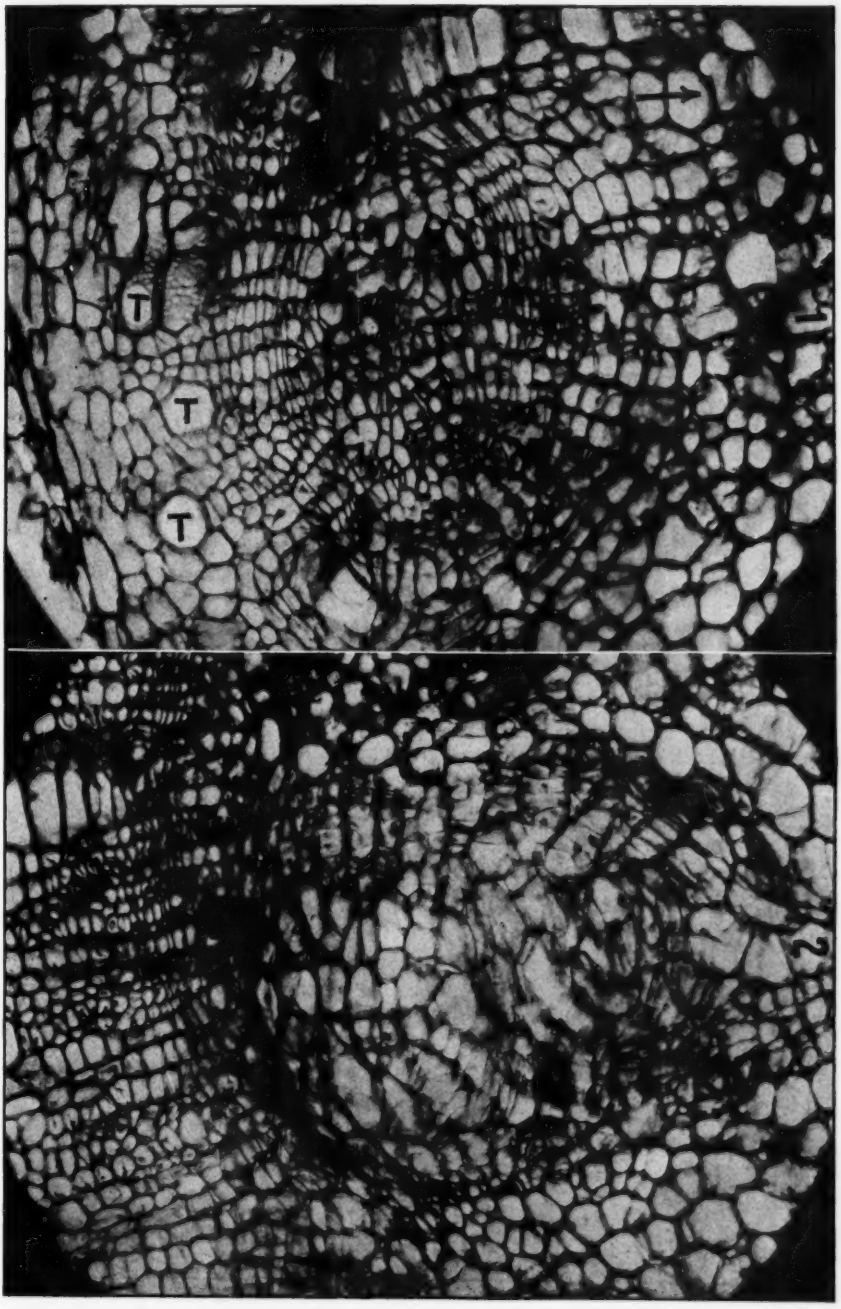
2.—From the same vascular ring, as figure 1, showing tracheæ lying at right angles to the longer axis of the stem, as often happens in crowngalls, and others properly oriented. Phloem at the top. Same magnification.

PLATE 40

1.—Cross section of second (pathological) xylem-phloem cylinder in Plate 37, figure 2, showing in the center a ring of cambial cells surrounding other cells (phloem). Below are tracheæ (*T*) some properly oriented, others lying at right angles to the long axis of the stem. The pith cavity lies in the direction of the arrow. ×160.

2.—Another swirl of cambium on the pith-cavity side of the second xylem-phloem ring in *Ricinus communis*. The xylem is in the lower part of the figure. ×160.

These cambial swirls appear to be concentric medullary bundles in the making Block 1265B.


T
T
T
1
2

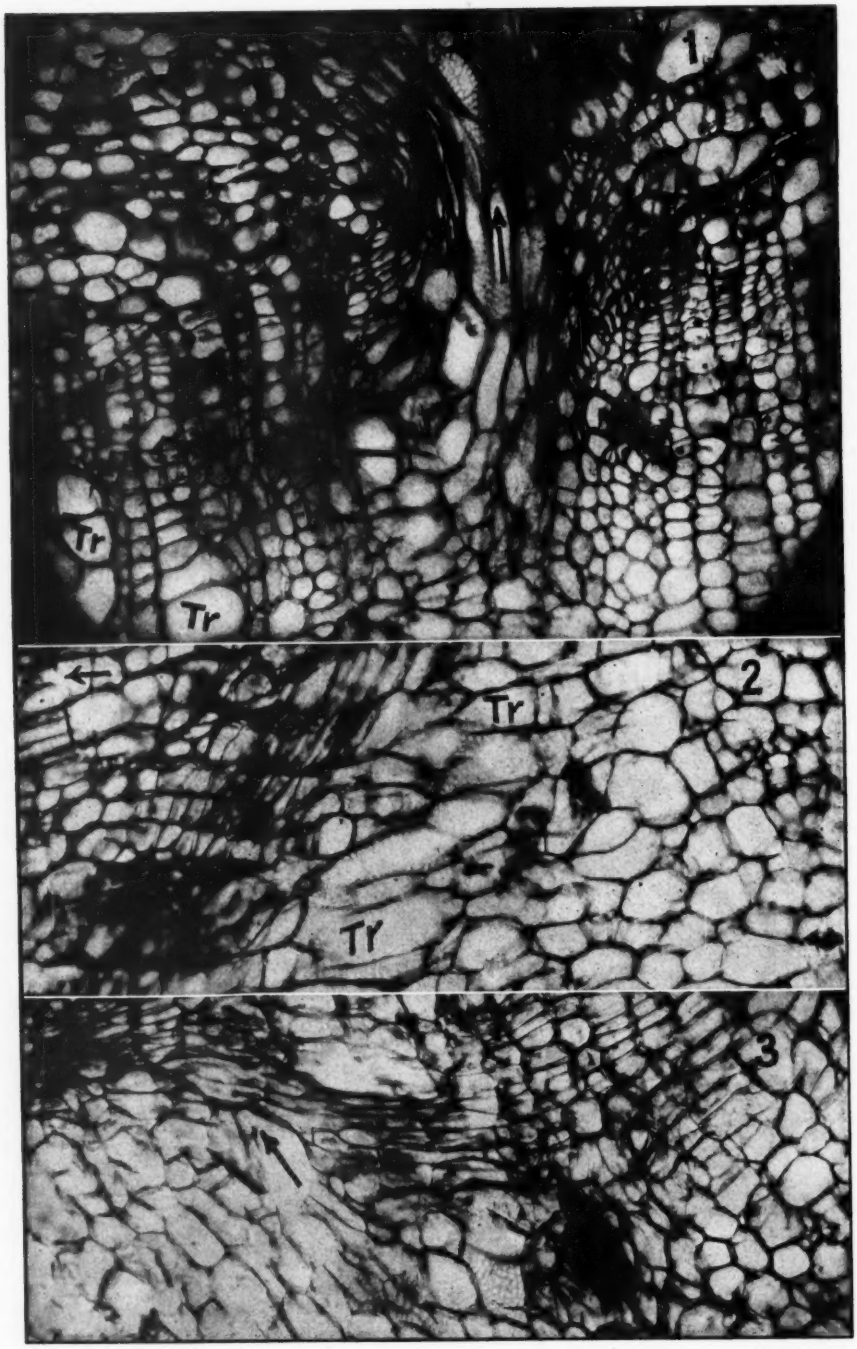

1
Tr
Tr
2
Tr
Tr
3

PLATE 41

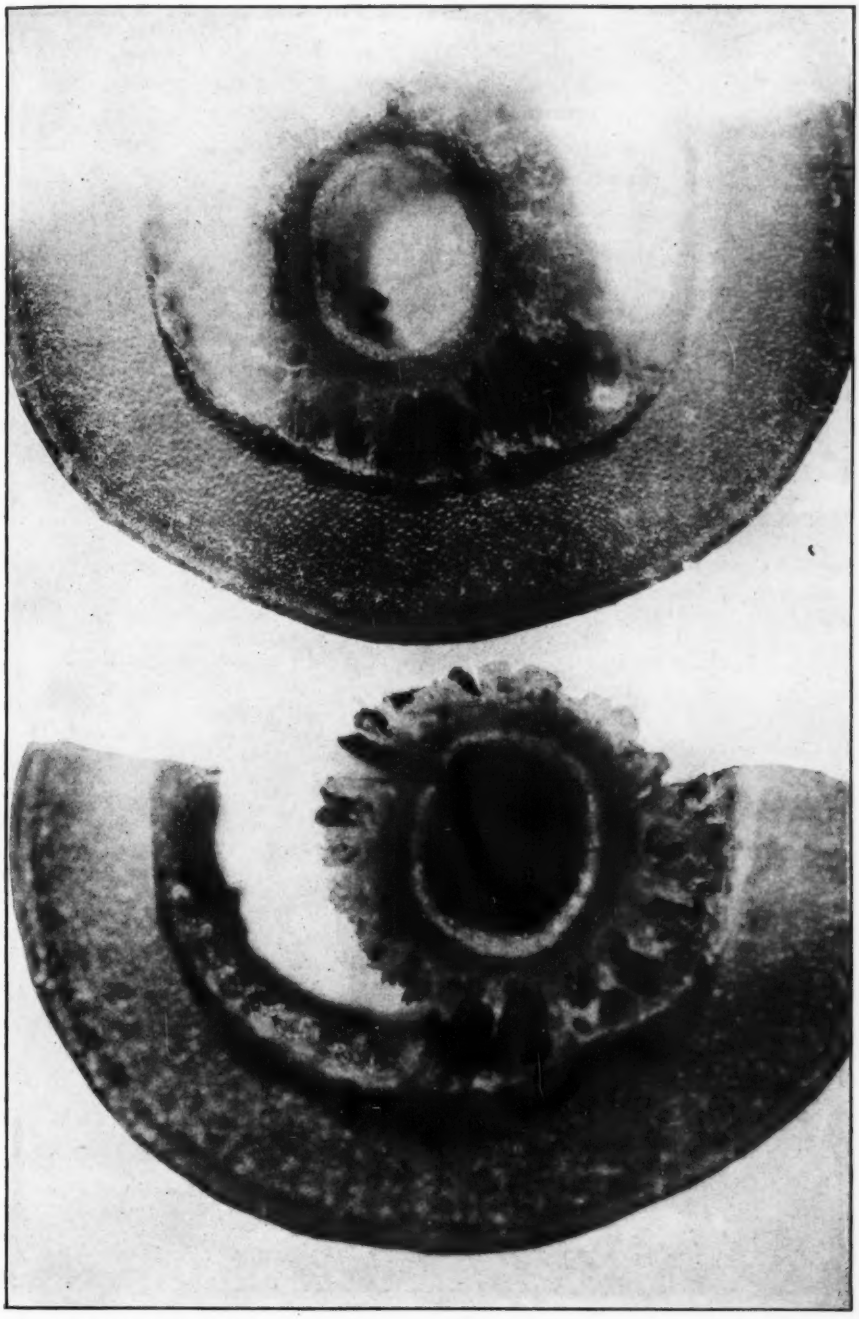
1. Second xylem-phloem ring in Plate 37, figure 2, showing a group of tracheæ, etc., lying at right angles to the long axis of the stem as in crowngall and sweeping out (direction of the arrow) into the pith (toward the pith cavity). Below, at either side, xylem-phloem normally oriented. *Tr*, tracheæ. ×160.

2. A portion of the fourth (imperfect) xylem-phloem ring in Plate 37, figure 2, showing tracheæ with cambium at their left, near the pith cavity, which is in the direction of the arrow, the second xylem-phloem being a little beyond this field at the right. ×160.

3. Like figure 2, but another part of the fourth xylem-phloem cylinder showing tracheæ, cambium, and sieve tubes. Same magnification. The pith cavity lies in the direction of the arrow.

PLATE 42

1, 2.—Cross sections of an internode of *Ricinus communis*, injected on July 22, 1916, (when young), with distilled water containing 5 per cent of dibasic ammonium phosphate. Much thickening of the pith, the inner pathological part of which has torn loose from the outer normal part. The cells in the region of the rupture were dead, but the remaining tissue of the separated pith cylinder, both the inner light ring of granulations and the middle dark ring, were fully alive. Photographed on August 3, 1916. ×8.



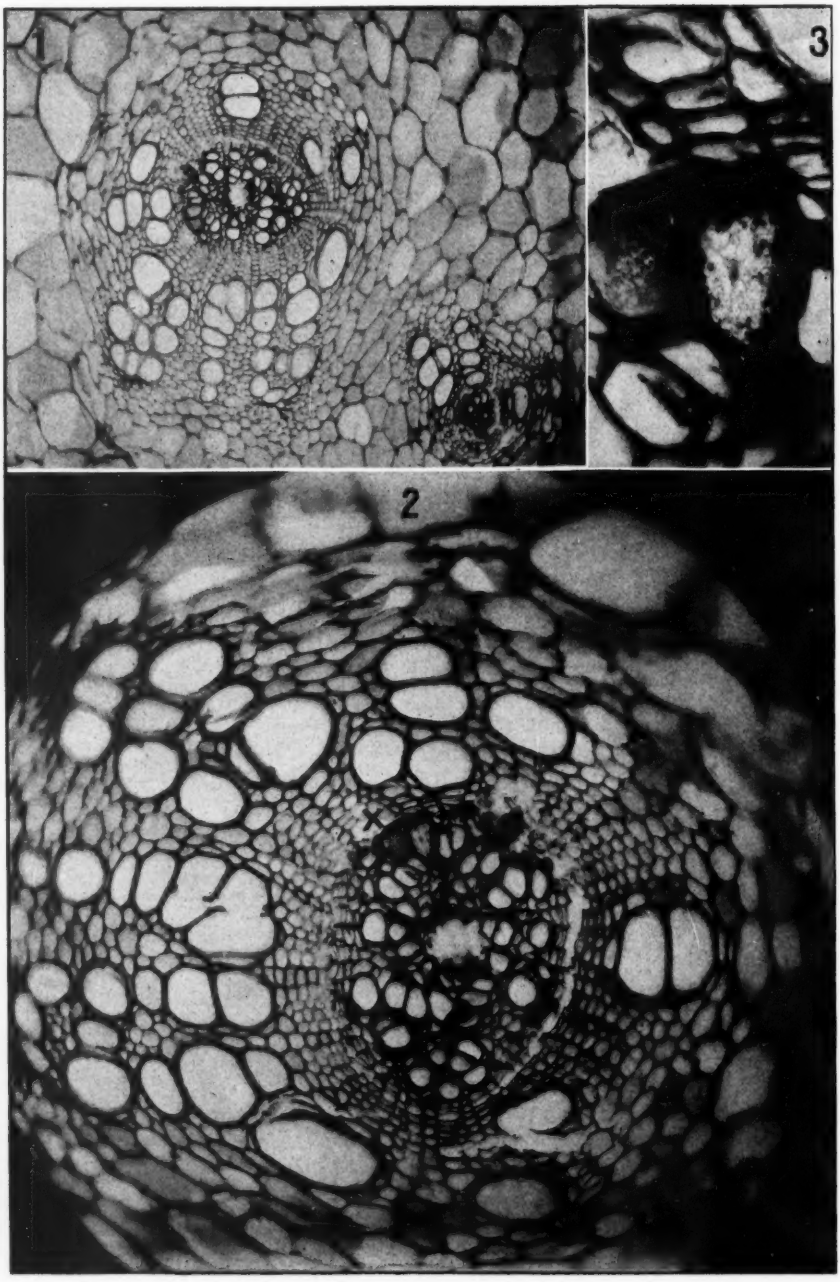

1
3
2

PLATE 43

1.—General view of the small displaced vascular strands (concentric medullary bundles) shown at *X* in Plate 20. The coarse-celled outer tissue is pith. ×75.

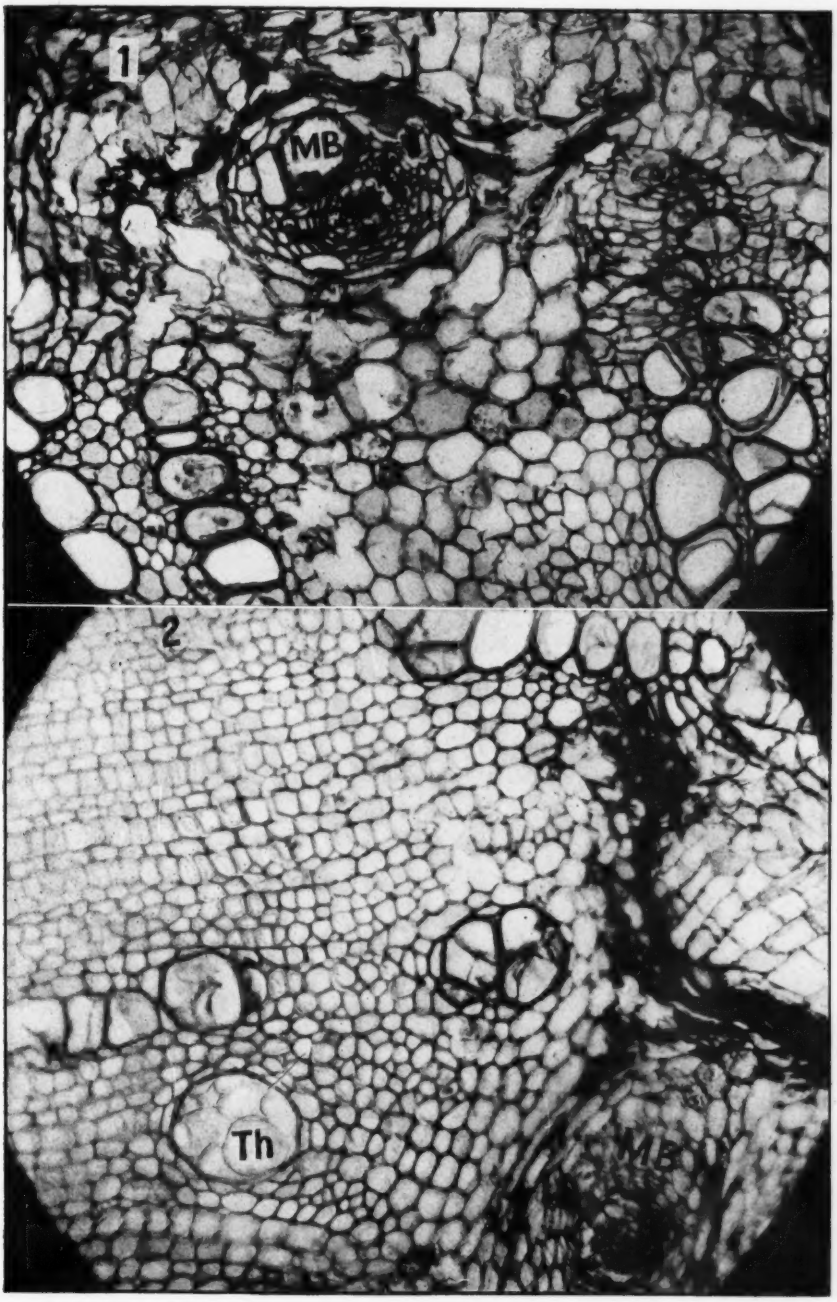
2.—Structure in cross section of the larger of the two strands in figure 1, xylem (spirals, tracheæ, etc.) on the periphery, cambium ring midway, phloem in the center. At *X* are two sieve plates. ×160.

3.—Sieve plates of figure 2 further enlarged. Block 1288. Slide 4. ×650. The perforations of these plates show quite well in the photograph, but are mostly lost in the overetched halftone.

PLATE 44

1.—Cross section of stem of *Ricinus communis* treated with ammonia (Pl. 5) showing the development of an abnormal (?) medullary bundle (*MB*) and its relation to the normal bundles. Pith above, inner part of woody ring below. ×160.

2.—Cross section of Ricinus stem treated with strong ammonia (Pl. 5) showing inner part of xylem, many tracheæ of which are filled with thyloses (*Th*) as in vascular bacterial diseases. At the right there is a little of the proliferated tissue, including one of the curious medullary bundles (*MB*) the center of which is phloem. Same magnification.


1
MB
2
Th
MB

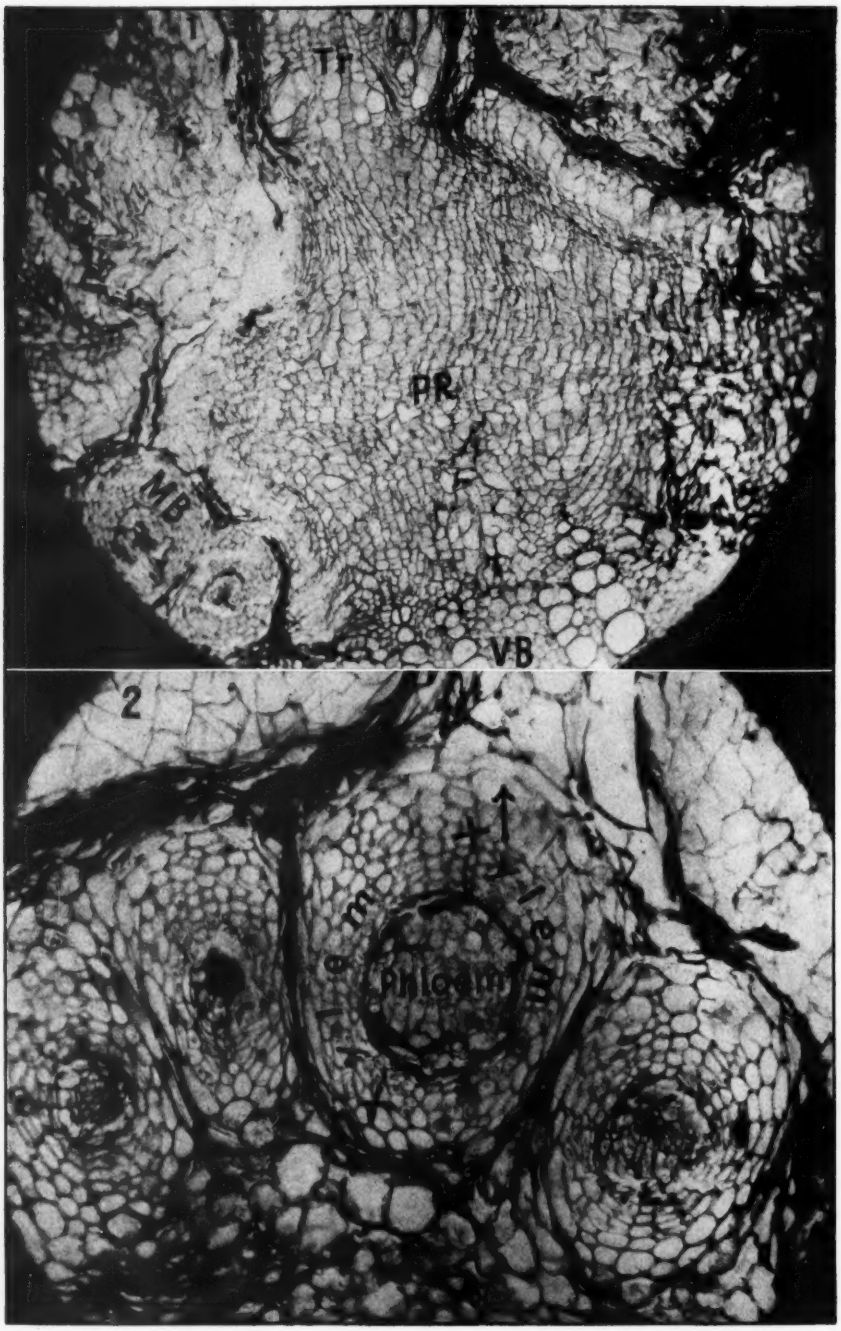

1
Tr
PR
MB
VB
2
Phloem
Xylem

PLATE 45

1.—Inner three-quarters of one of the middle proliferations on Plate 5, showing its origin from the inner face of the vascular bundles (*VB*) with tracheæ and sieve tubes of its own at *Tr*. At *MB* are two concentric medullary bundles. ×70.

2.—Ricinus stem treated with strong ammonia (Pl. 5) showing four whorls of vascular tissue (concentric medullary bundles) similar to those occurring in crowngalls: i. e., the central tissue is phloem and the outermost cylinder is xylem. The pith cavity is in the direction of the arrow. Below is a branch gap (?). ×160. Compare with Plates 40, 43, and 65.

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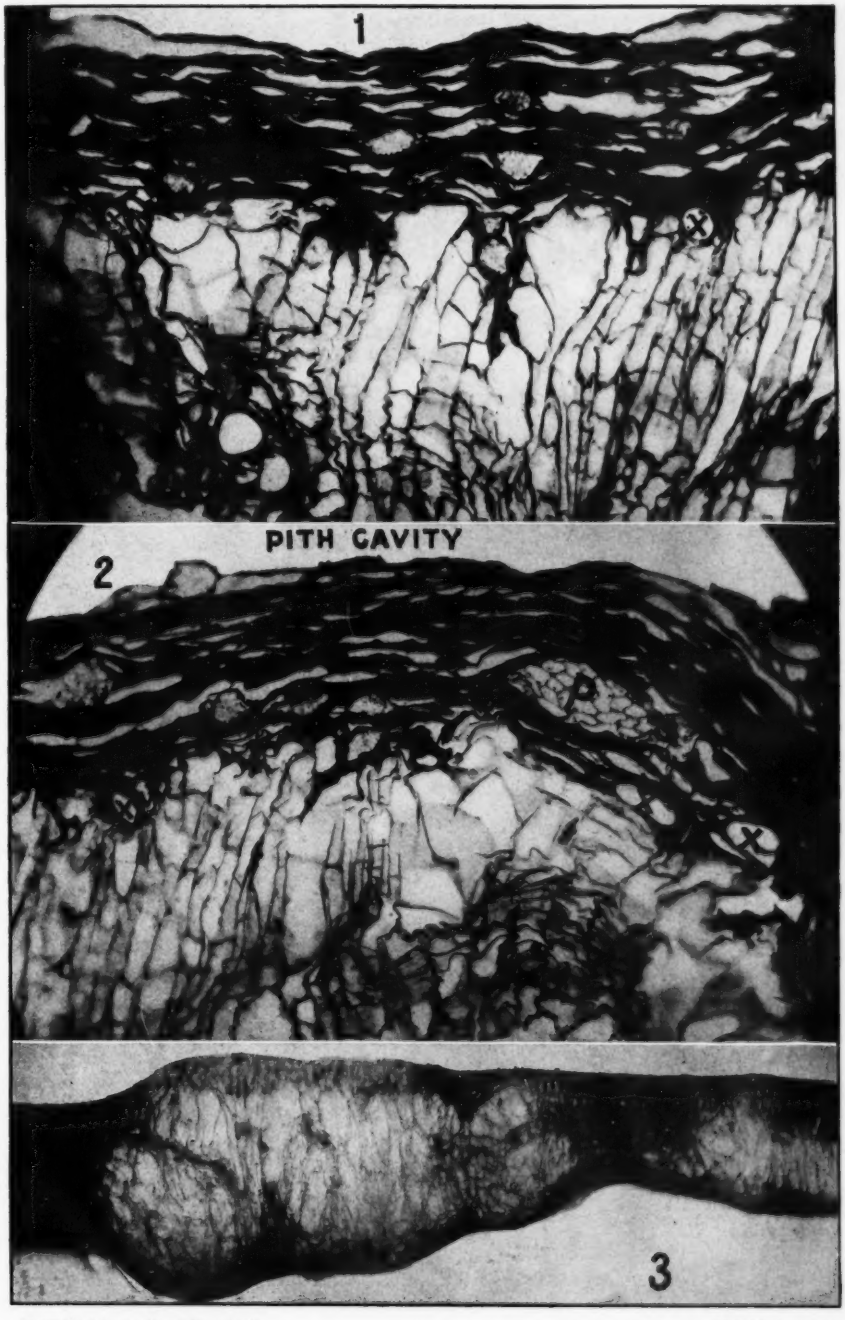
PLATE 46

1, 2.—Cross sections of the killed tissue (pith) at one side of the proliferations shown on Plate 5. Above in each are rows of collapsed dead pith cells containing many crystals of calcium oxalate. Below are tissues which have proliferated from the inner face of the bundle sheath. Below at the left, in figure 1, bundle vessels are included. In figure 2, which joins on to the right of figure 1, the section cuts above the vessels of the bundles. In the middle of each section immediately under the killed (black) tissue (at *XX*) are vessels, and at *P* a little mass of tissue interpreted as new phloem, but no sieve plates were found in it. This phloem and the new xylem vessels must have formed in advance of the death of the tissues surrounding it (it was not itself dead tissue) and further growth stopped by the deeper killing in of the pith tissues. A similar small mass of phloem (also subtended by spiral vessels) is visible in the same relative position in sections made from the other side of the proliferations shown on Plate 5. This is, I believe, the interrupted beginning of a second xylem-phloem ring. ×160.

3.—Cross section of cauliflower leaf, showing intumescences formed after exposure to vapor liberated from one-half gm. of secondary methylamin chloride by adding 10 c. c. of a 15 per cent solution of sodium hydrate. A free-hand unstained section of one of the intumescences shown on Plate 62. Time of exposure to the vapor, 30 minutes. Temperature, 18°C. Date of collection, nine days after exposure. The section shows local thickening of the tissues with destruction of the chlorophyll. The dark parts of the section were green. ×75.


1
PITH CAVITY
2
P
3

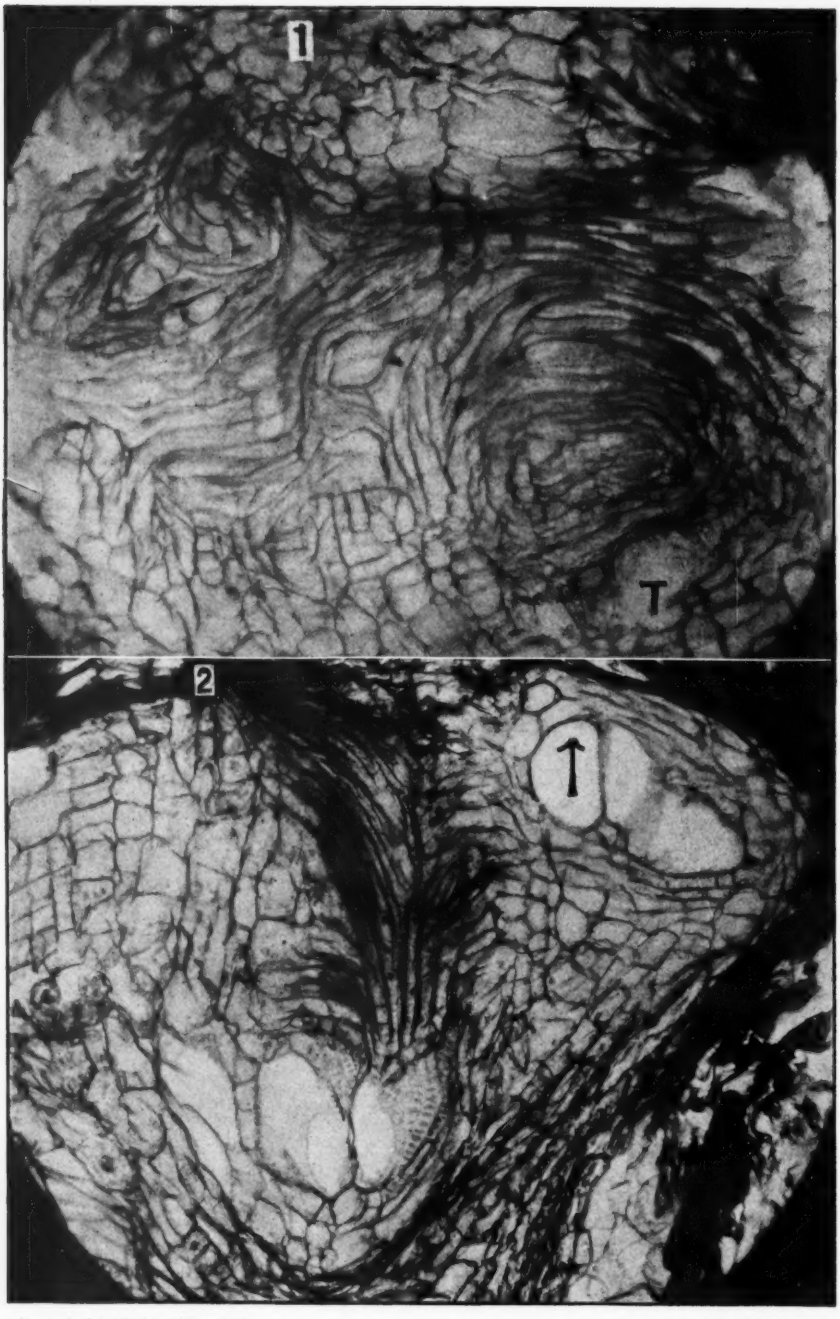

1
T
2

PLATE 47

1.—Vertical section of outer part of the proliferations just below the surface shown on Plate 5. Here are well-developed tracheæ (at *T*) and long, twisted cells (incipient vessels) strikingly suggestive of crowngall irregularities. ×160.

2.—Vertical section in same region as figure 1, but at another level, showing distorted vascular bundles in the new growth. The arrow shows the direction of the pith cavity, and the tissue at the top shows the beginning of the outermost proliferations—that is, those into the pith cavity. ×160.

PLATE 48

Portion of under surface of a cauliflower leaf showing intumescences due to vapor of ammonia. Time, 96 hours. The plant was exposed for 15 minutes at 20° C. to the vapor from 0.5 c. c. of aqua ammonia (sp. gr. 0.90) in 10½ cubic feet of air space, after which the plants were returned to the hothouse. The exposure was made at 11 a. m., and where no eruptions have appeared it may be assumed that the stomata were closed, particularly as the leaf was young. All of the outgrowths are on the lower face of the leaf, which is more abundantly provided with stomata than the upper surface, although there are also a great many on the latter. All of the smaller intumescences are still covered by a stoma-bearing epidermis. Exposed on August 28, 1916. Photographed on September 1. ×5.

1
2

PLATE 49

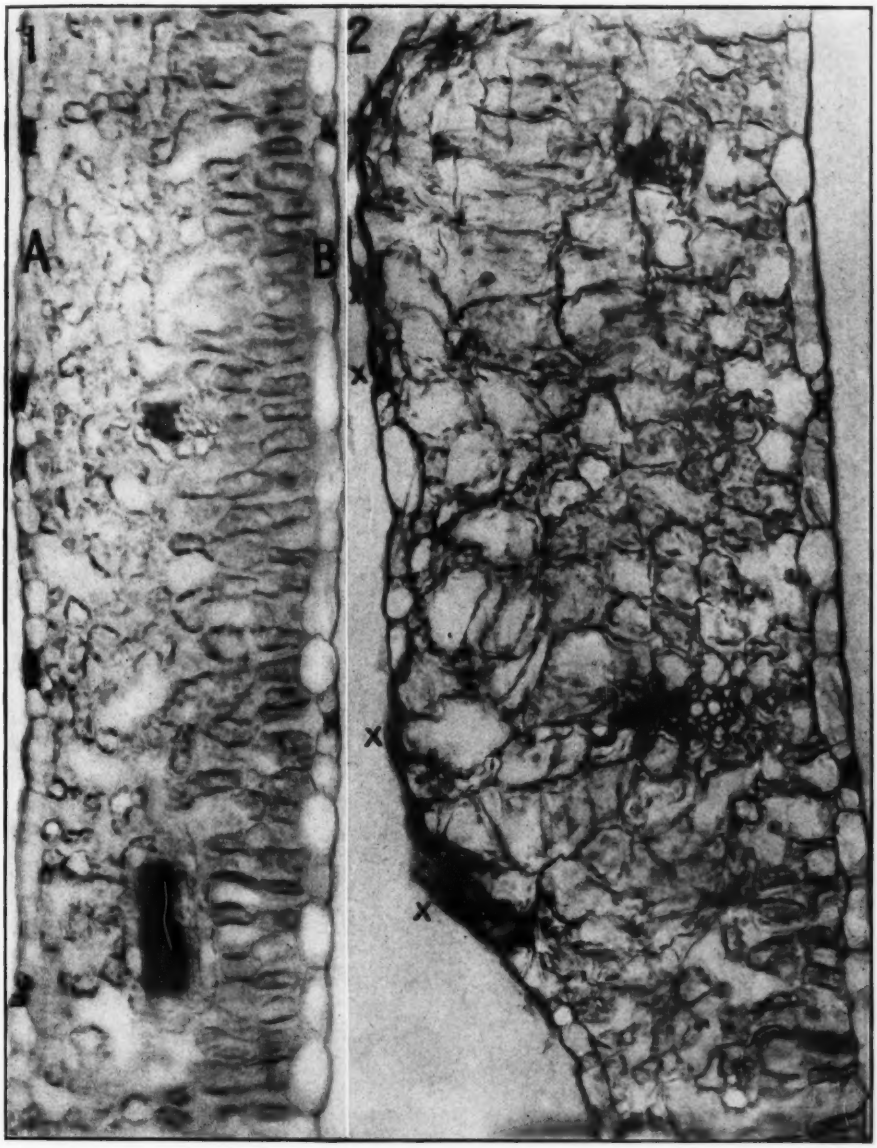
1.—Under surface of a cauliflower leaf from the same series as Plate 48 but nine days after 15 minutes' exposure to vapor of ammonia, and not from the same plant. Galls much larger and epidermis now ruptured in many of them. Most of the intumescences are on the under surface, and they occur in clusters. Probably the stomata on the upper surface of leaves, being exposed to the sun, close earlier in the day than those on the under surface, and those on the under surface undoubtedly close irregularly according to the needs of the various areas of the leaf. (For further evidence of this see Pl. 55.) The leaves became mottled light and dark green soon after exposure, but the phenomenon was fugitive—that is, two hours later they appeared to be normal. Photographed on September 6, 1916. × 5.

2.—Same series as figure 1, but at the end of 18 days, and from the upper surface of a leaf. Photographed on September 1, 1916. ×3.

PLATE 50

1.—Cross section of a normal cauliflower leaf. *A*, Lower surface; *B*, upper surface. Seven stomata are visible. In the center are two small vascular bundles, one cut longitudinally, the other crosswise. Section stained with methyl green and acid fuchsin. × 210.

2.—Early stage (third day) of cauliflower intumescence due to vapor of ammonia. The lower epidermis still covers the growing tumor and five stomata are visible in it (at *XX*). Same magnification and same leaf as in figure 1. Tumor formed out of the loose parenchyma. Particular attention is called to the increased size of the cells and the reduction of intercellular spaces in the intumescence, also to changes in the palisade tissue.


1
A
B
2
X
X
X

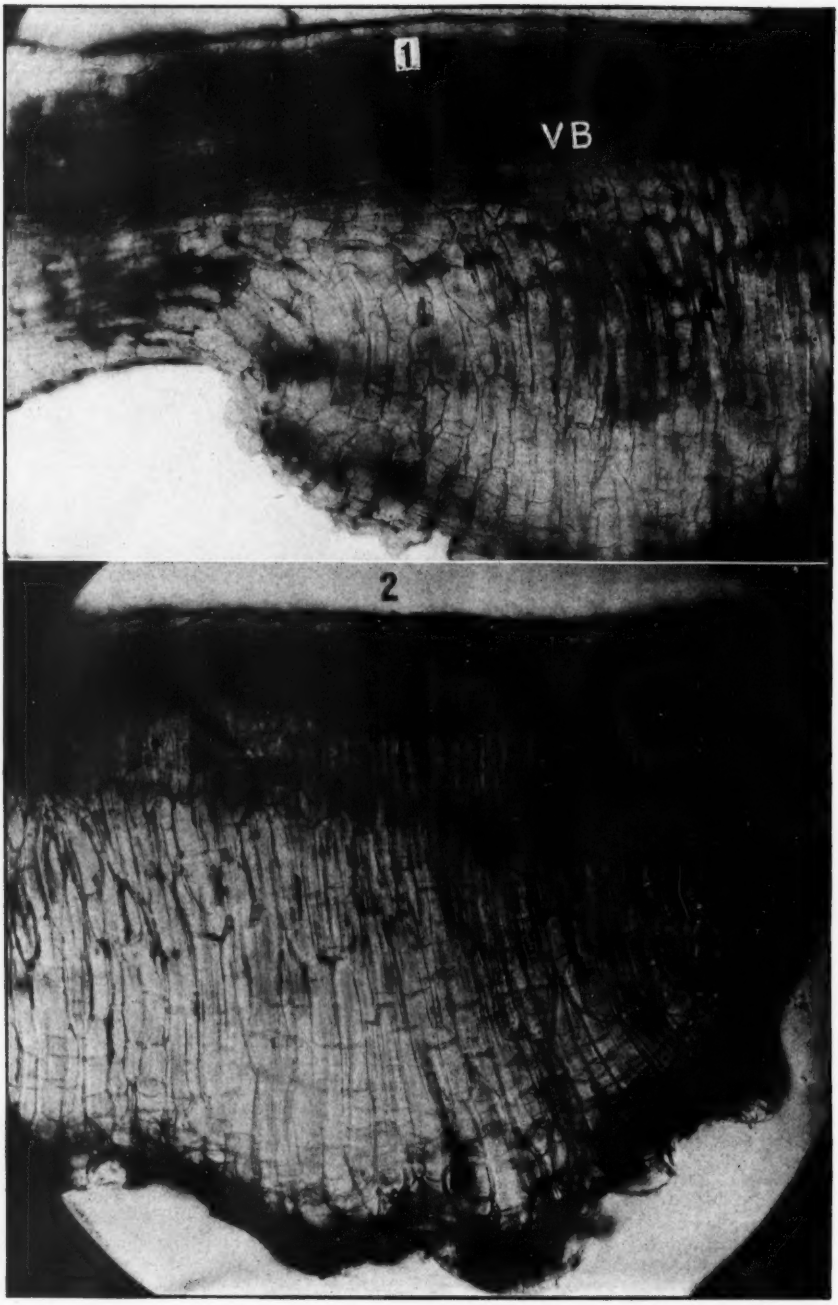
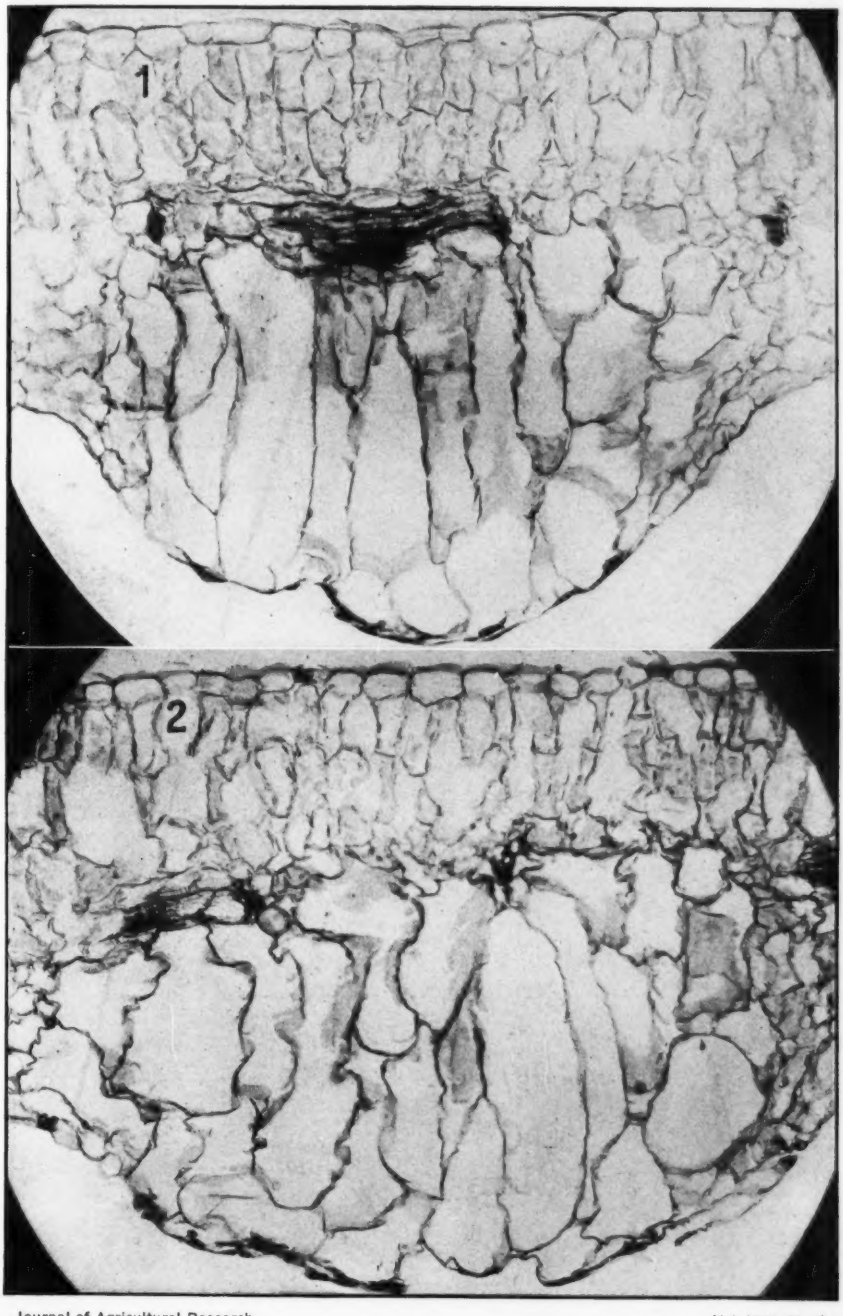

1
VB
2

PLATE 51

1, 2.—Free-hand unstained sections (water mounts) from old cauliflower intumescences caused by vapor of ammonia. The proliferation, which is much more compact than the normal tissue, is entirely or almost entirely from the loose parenchyma below the palisade tissue. The latter contains chloroplasts, but the intumescence does not. The vascular system (fig. 1 *VB*) appears to have been very little disturbed. Same series as Plate 50, figure 2, but much older. Exposed on August 28, 1916, sectioned and photographed on September 30. Most of the intumescences had shriveled by the end of the eighth week—that is, the stimulus was exhausted. ×160.

PLATE 52

1, 2.—Vertical sections of two ammonia-vapor intumescences on a cauliflower leaf showing an enormous hypertrophy of cells and no hyperplasia. The sections are from stained material which was fixed nine days after exposure to the vapor. Intumescences developed wholly from the cells of the loose parenchyma—that is, palisade tissue and vessels not involved. The volume of the largest cells in the tumor is a hundred times that of the normal cells out of which they have developed. These sections may be contrasted with those on Plates 51 and 57 to 61 which show hyperplasia. ×160.


1
2

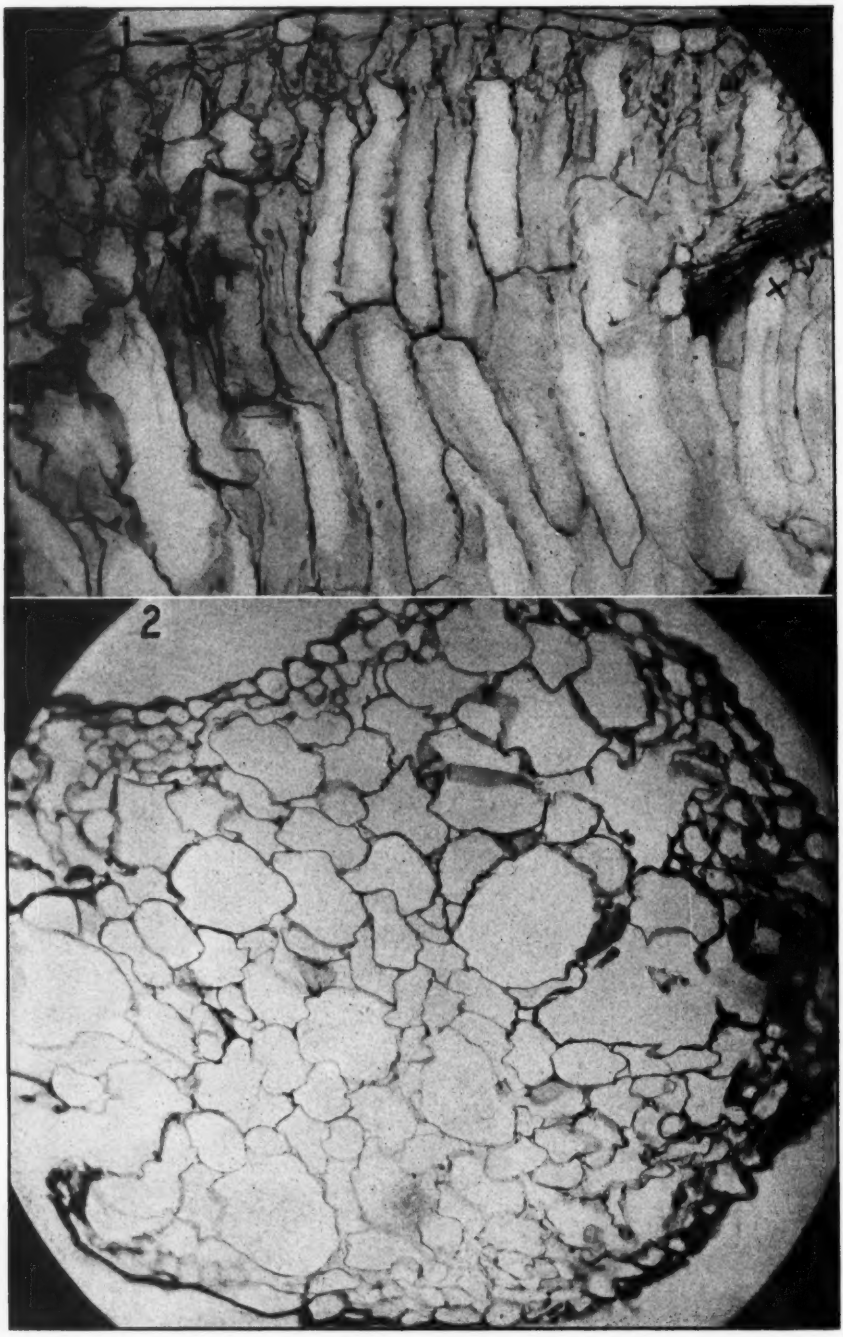

1
2

PLATE 53

1.—Like Plate 52 but here the palisade tissue is also involved. No evidence of hyperplasia nor much of ingrowths of vascular tissue (*X*). Two rows of cells at the lower surface of tumor omitted. Same magnification as Plate 52 (×160).

2.—Cross section of an ammonia intumescence above the level of the under surface of the leaf. Contrast with Plate 58, figures 1 to 4, which have the same magnification. Time, 9 days. × 160.

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PLATE 54

1, 2.—Cross sections through the deeper tissues of two ammonia intumescences in a cauliflower leaf showing that even in the depths they preserve their hypertrophic character. Time, nine days. The intumescences are surrounded by the normal tissues of the leaf. At *VB* are vascular bundles. Figure 1, × 160; figure 2, × 75.

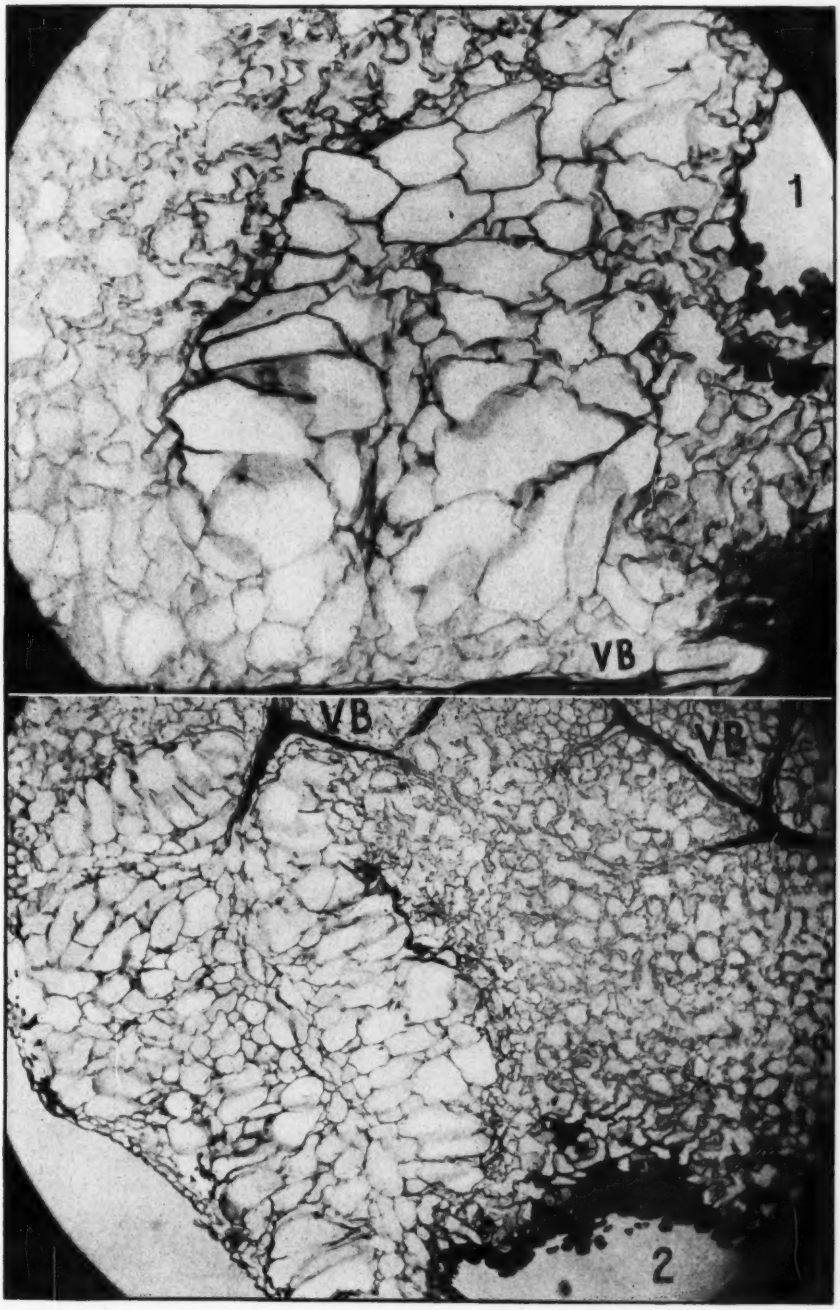
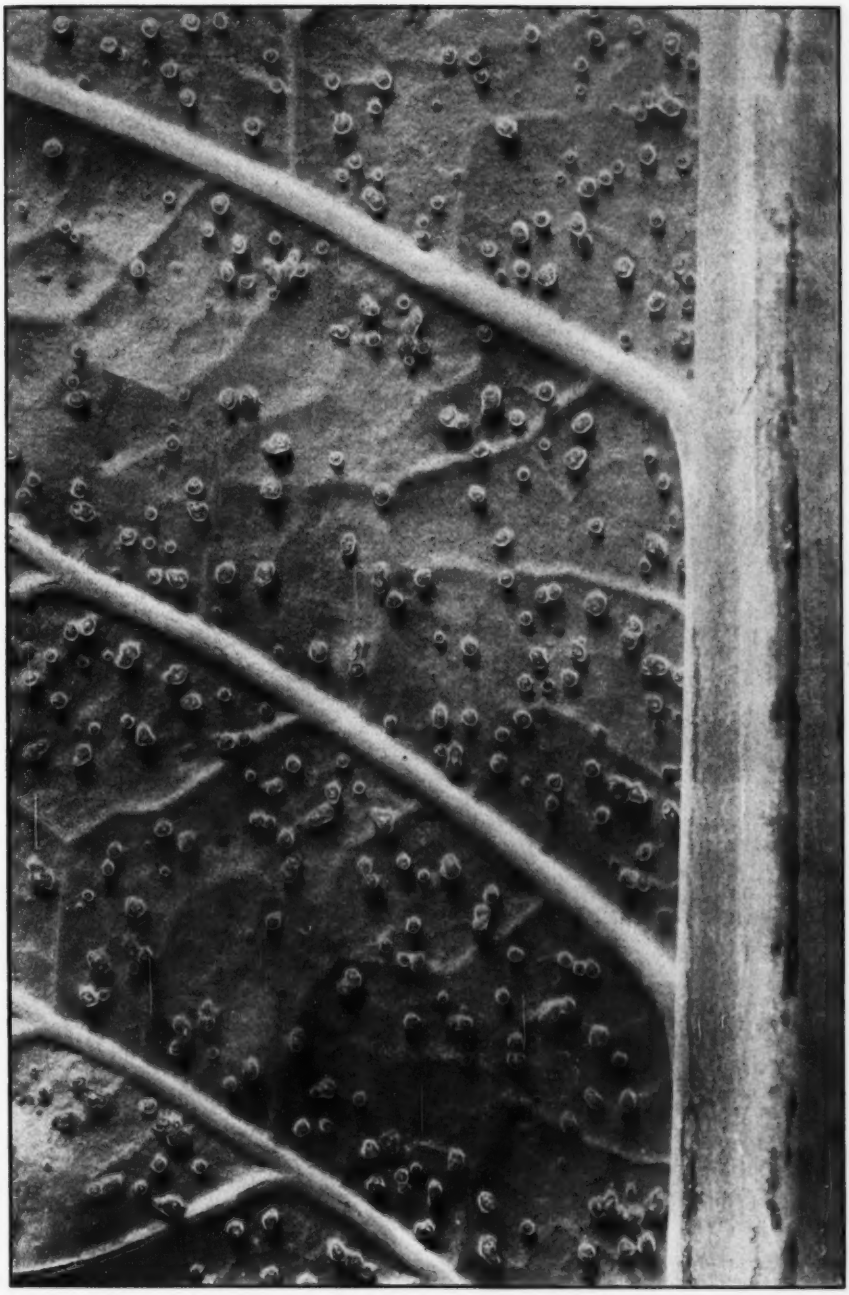

1
VB
VB
VB
2

PLATE 55

Cauliflower leaf showing killing effect of vapor of mixed ethyl alcohol and acetic acid (Carnoy's fixing fluid—three-fourths absolute alcohol, one-fourth glacial acetic acid) on maturer leaves of the same plant as Plate 56. The stomata were open presumably in the particular areas which have been killed (the white parts) and not in the other parts. The local killing effect was visible immediately. No intumescences appeared on such leaves, not even around the margins of the white spots. The other parts of the leaf remained normal in color and function. Exposed on September 21, 1916. Photographed on September 28. ×1¼.

PLATE 56

Under surface of a cauliflower leaf showing intumescences produced by the vapor of mixed ethyl alcohol and acetic acid (crowngall products). Leaf young when exposed and the stomata closed or nearly closed, so that only a very little of the vapor could enter. The plants were inclosed in 10½ cubic feet of air space at 20° C. and exposed for 30 minutes on September 21, 1916, at 1 p. m., to the vapors from 10 c. c. of the fluid in a porcelain capsule floating in a dish of water at 65° C. Only a small amount of the fluid was vaporized but enough to be smelled. Older leaves (half grown) were killed quickly in many irregular spots (Pl. 55), but leaves of this age did not show any immediate injury. The height of the intumescences may be judged by their shadows. Occasionally several are fused. Some are too small to show on the plate. None developed on the midrib or on the main veins and none or very few on the upper surface. Other leaves, where a greater number of stomata may be assumed to have been slightly open, showed hundreds of tiny coalescent intumescences, the under surface in large patches being one mass of eruptions. Photographed on September 28. ×5.



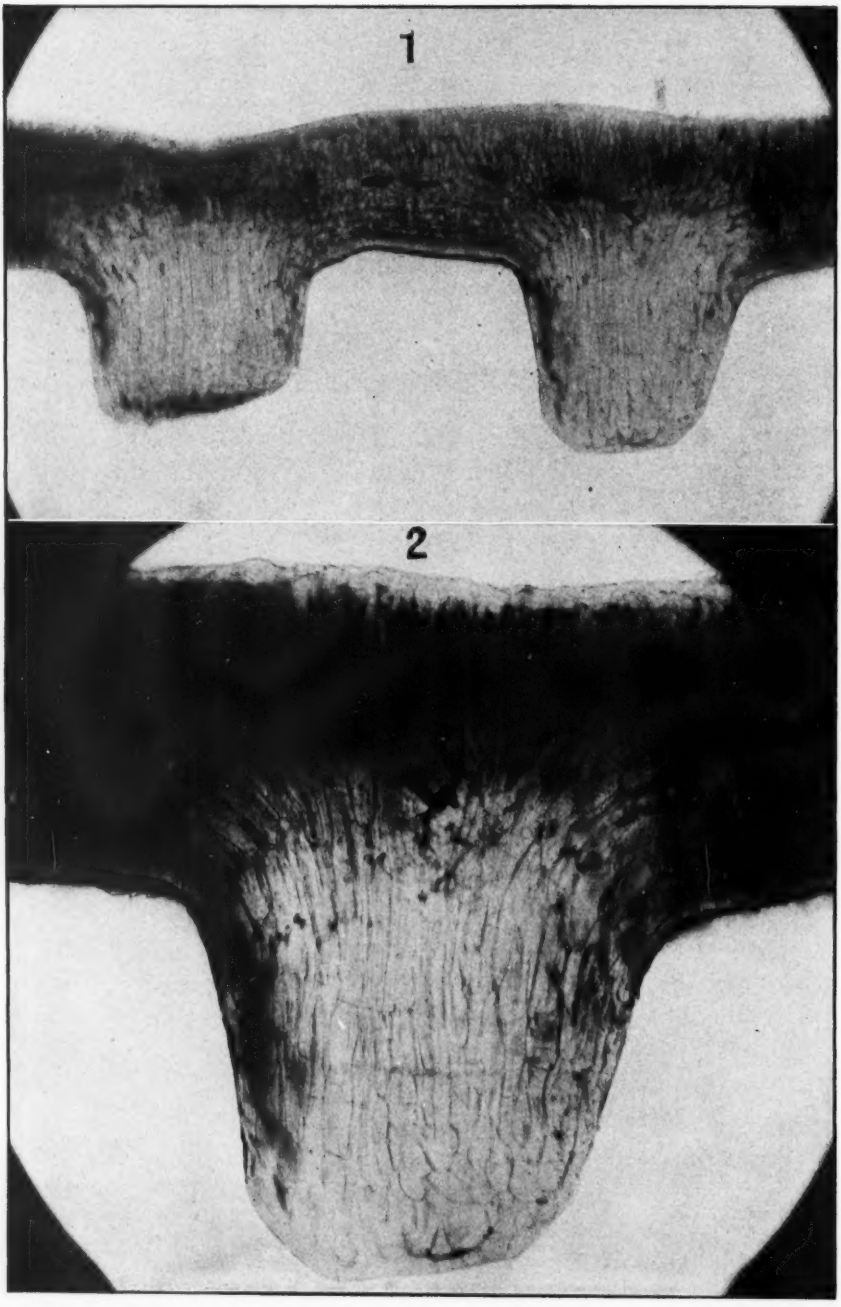

1
2

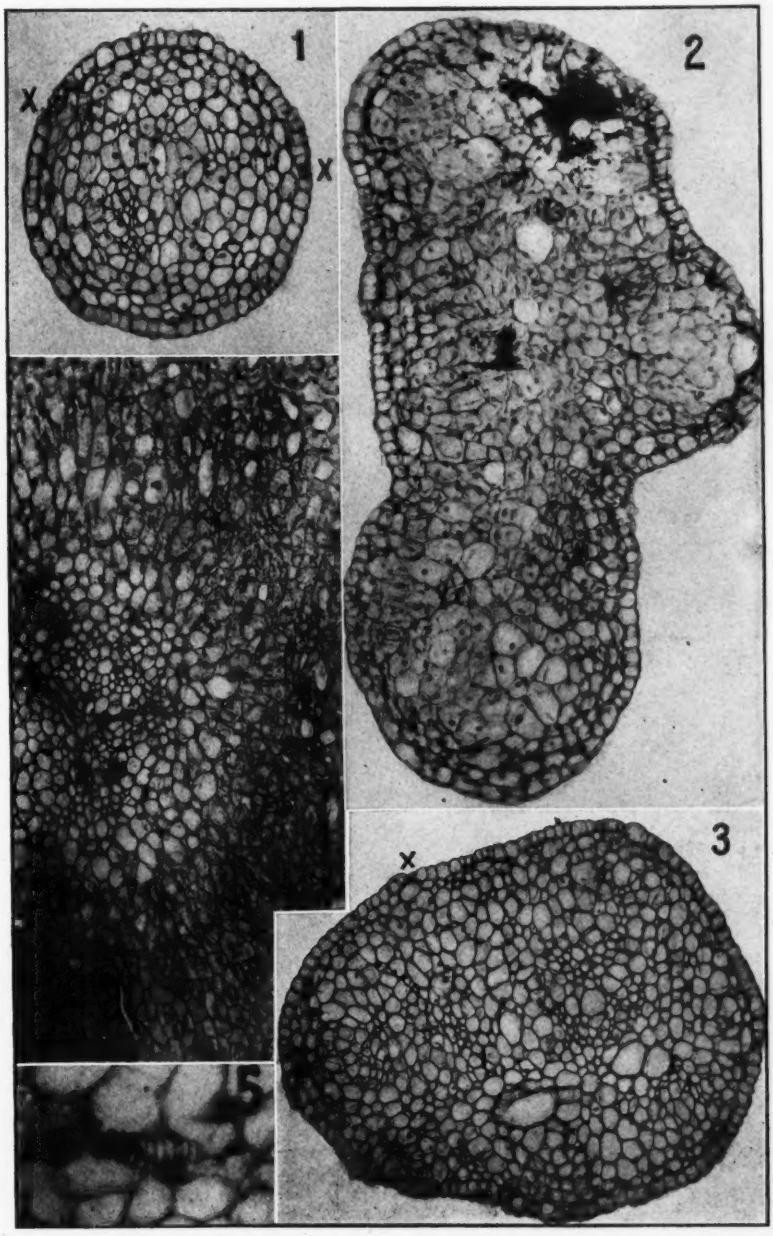
PLATE 57

1.—Cauliflower intumescences due to vapor of mixed ethyl alcohol and acetic acid (Carnoy's fixative). Free-hand unstained section (water mount) from the leaf shown on Plate 56. The growths are unlike many of those due to vapor of ammonia—that is, they are all hyperplasias. The tumor tissue is much more compact than the loose parenchyma from which it has originated, is free from chlorophyll, and is not endowed with continuous growth. × 75.

2.—This is an enlargement of the right-hand tumor shown in figure 1. The darker parts, at least in the normal portion, represent chlorophyll-bearing tissue. Exposed on September 21, 1916. Photographed on September 28. × 160.

PLATE 58

1 to 5.—Cross sections from fixed and stained portions of the little tumors which developed on the leaf shown in Plate 56. Each one is bounded by an epidermis, and in places stomata are visible, as at *X*. The smaller cells in each, best seen in figure 3, are cross sections of incipient vascular bundles. Figure 4 is a section just below (inside) the lower surface of the leaf. At the bottom is epidermis showing stomata. On the right is unchanged loose tissue of the leaf out of which the hyperplasia has developed. This shows large intercellular spaces. In the middle at the left is the compact tissue of the tumor, while above it is transition tissue. Observe absence of intercellular spaces in the tumor and the beginnings of spiral vessels. × 160. Figure 5 shows spiral vessels enlarged from the center of the tumor in figure 4. ×640.


1
X
X
2
3
X
5

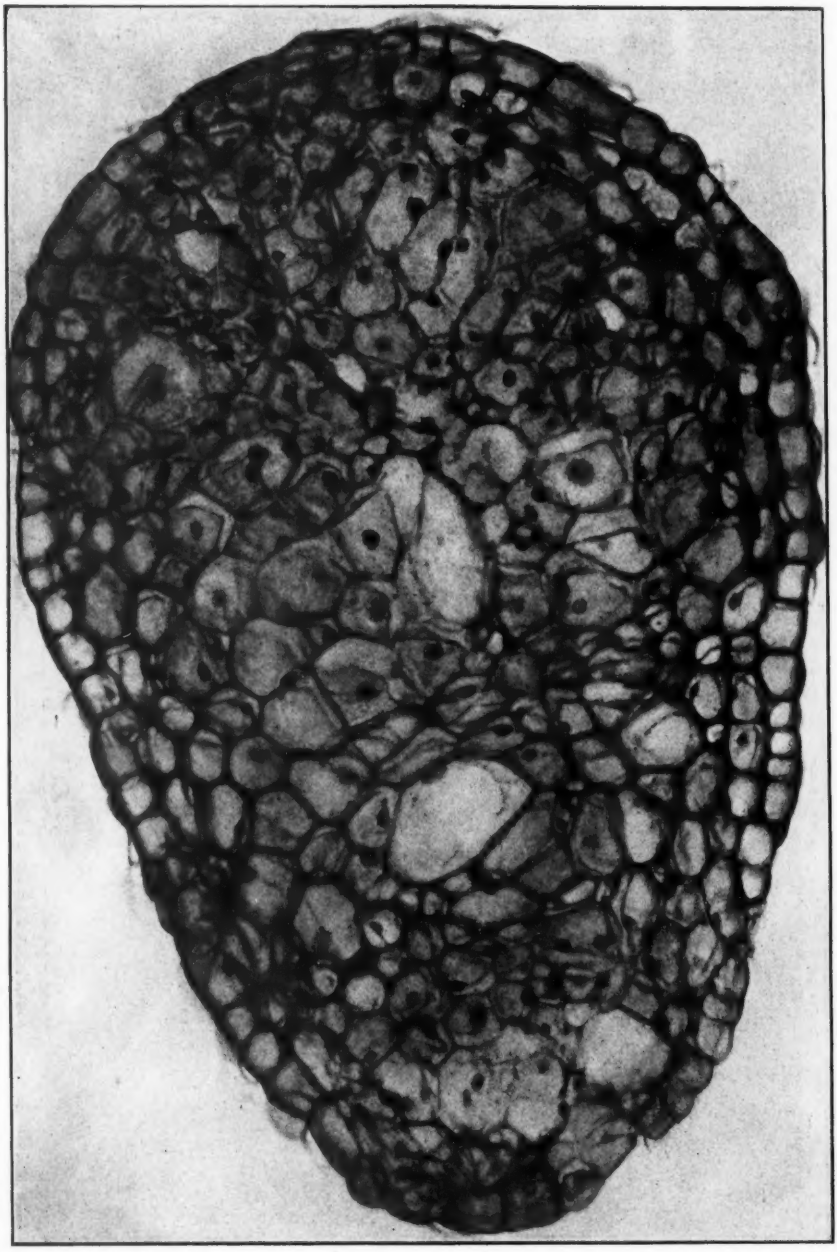


PLATE 59

Cross section of one of the intumescences of Plate 56 further enlarged to show mixed hypertrophy and hyperplasia as in crowngall. The round, dark spots in the cells are nuclei. This had the same initial magnification as Plate 58, figures 1 to 4, but the photograph was enlarged three times by the engraver. ×480 (circa).

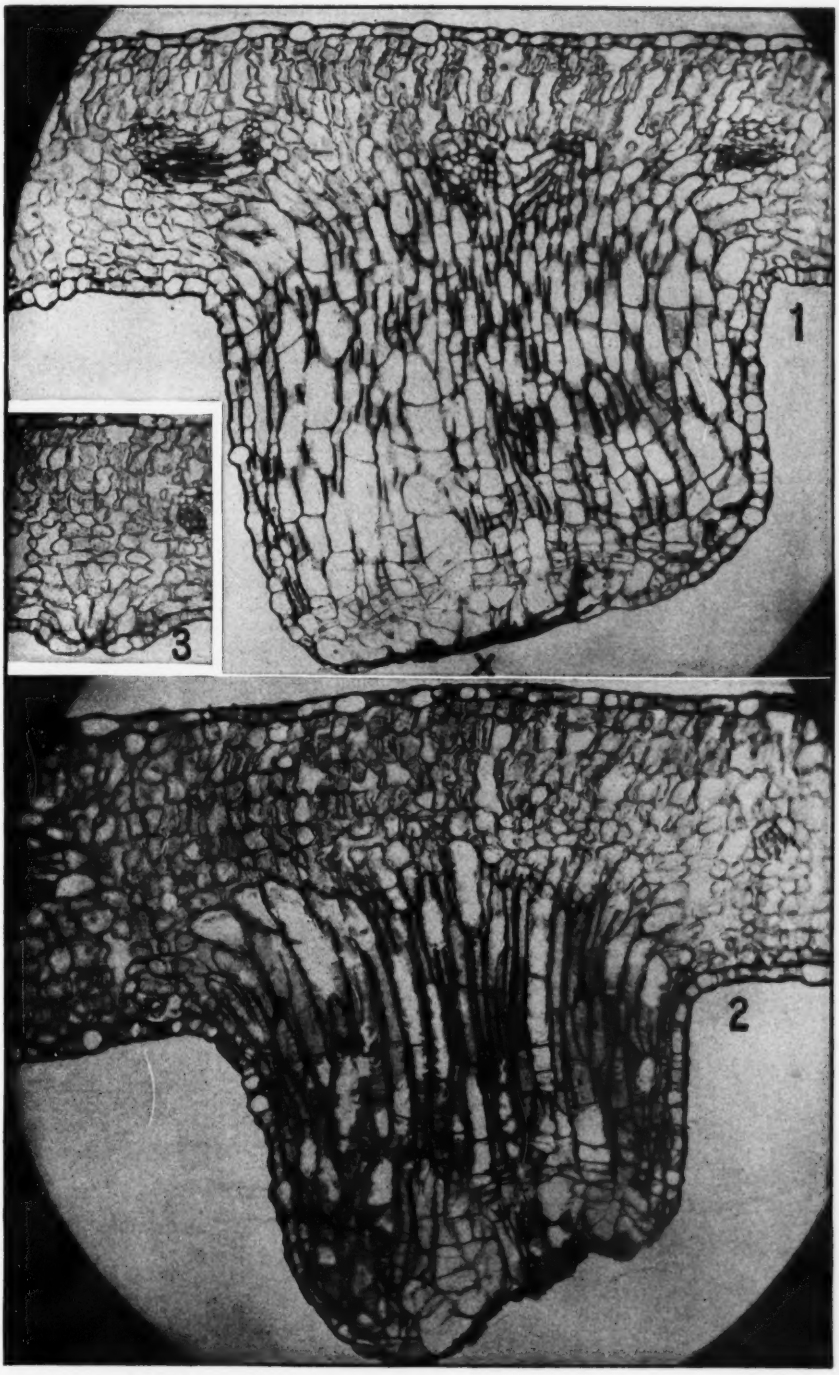
PLATE 60

Vertical sections from fixed and stained portions of cauliflower intumescences shown on Plate 56 and in cross section on Plates 58 and 59. Time, 7 days. Upper surface of leaf at top.

1.—Middle vascular bundles giving off incipient vessels to the tumor. The epidermis is dead at *X*, and a cork layer has formed under it. × 160.

2.—Sieve tubes and incipient xylem vessels developing. Here again the epidermis covering the top of the hyperplasia is dead, and cork has formed. × 160.

3.—Section through the middle of an intumescence which received a *minimum* of stimulation and in which only the epidermis and two layers of cells immediately under it have responded. Time, 7 days. Many such abortive tumors can be seen on the surface of the photograph from which Plate 56 was made and some of them also in the half-tone. ×160.


1
2
3

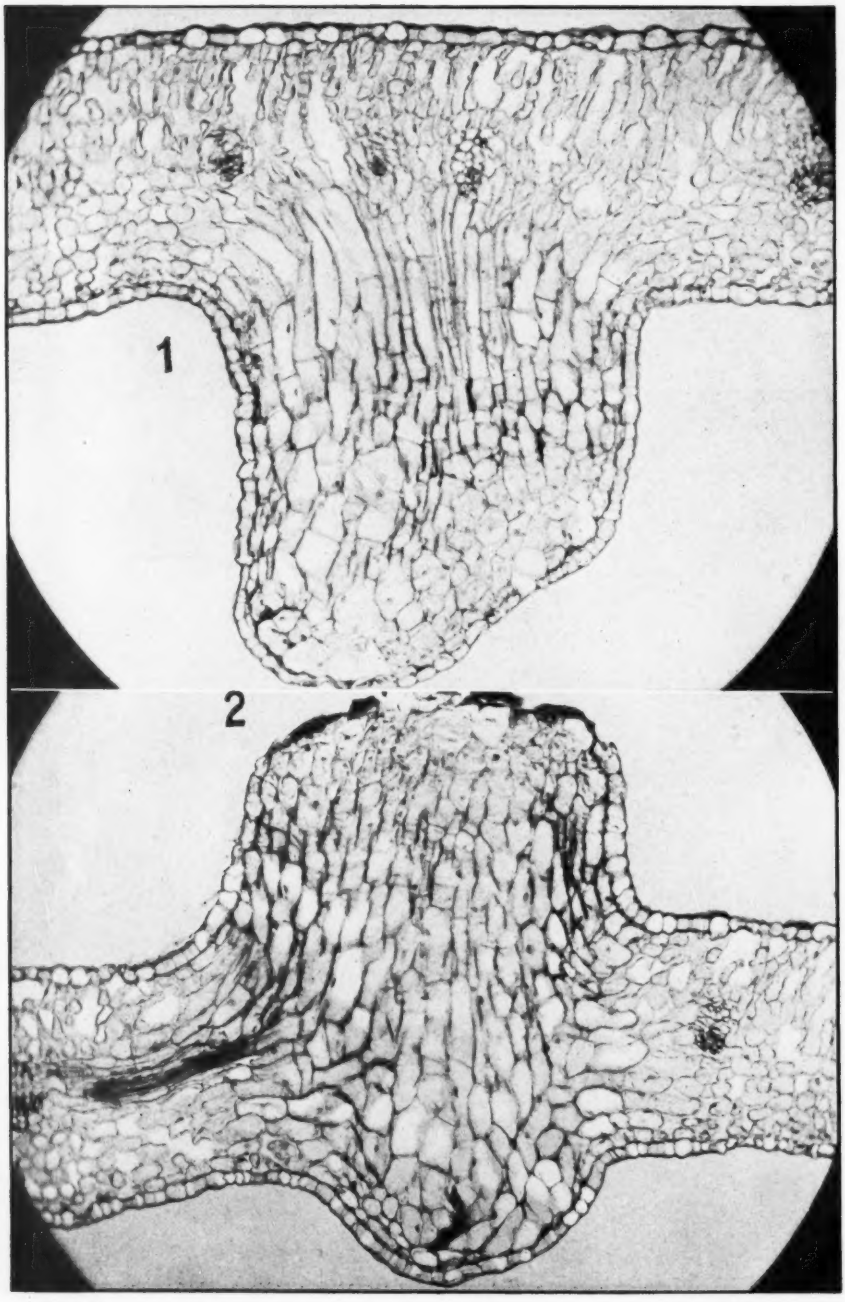

1
2

PLATE 61

1.—Like figures 1 and 2 of Plate 60, but with the intumescence still covered by an epidermis and free from cork. Incipient vessels can be seen entering the tumor from the leaf bundles. Upper surface of leaf at the top. × 160.

2.—Alcohol-acetic-acid intumescence in which entire thickness of the cauliflower leaf is involved. In this instance the larger part of the growth is from the palisade tissue (at the top). × 160.

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PLATE 62

1, 2.—Intumescences formed on a cauliflower leaf exposed 30 minutes in 10 1/2 cubic feet of air space to vapor liberated from one-half gm. of secondary methylamin chlorid by adding 10 c. c. of a 15 per cent solution of sodium hydrate. Temperature, 18° C. Time, 2 p. m. Collected nine days after exposure.

1.—Under surface of leaf corresponding to figure 2.

2.—Upper surface of same leaf. Many of these intumescences involved the whole thickness of the leaf; others appeared only on the under side, or only on the upper side. In figure 1 the unruptured swellings at *X*, *X*, correspond to ruptured tumors on the other surface of the leaf. × 3 1/2 nearly.

Inasmuch as it is probably more or less difficult for the chemist to obtain an amin chlorid entirely free from ammonium chlorid it is possible that we have here to deal with mixed stimuli. This can not be stated positively until further experiments are made. My reason for thinking so is that in addition to the fishy odor of the dimethylamin, the odor of ammonia was also detected, especially when the nose was brought close to the capsule containing the mixture.

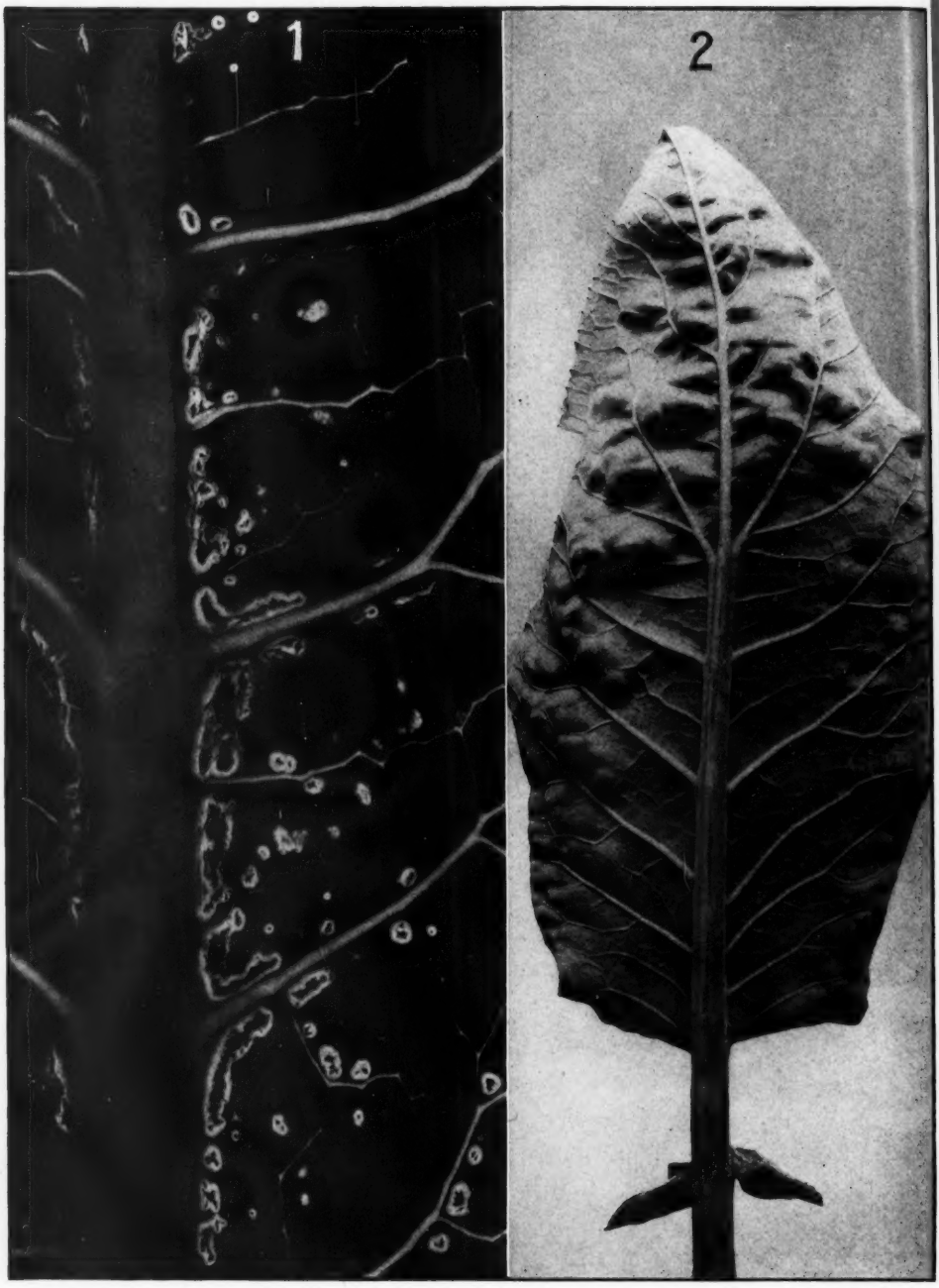
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PLATE 63

1.—Same as Plate 62 but photographed by transmitted light to show the opaque centers of the intumescences due to death of tissues and the chlorophyll-free translucent borders which allowed the light to shine through readily, and therefore are white in the photograph. All the larger intumescences are ruptured. ×3 1/2 nearly.

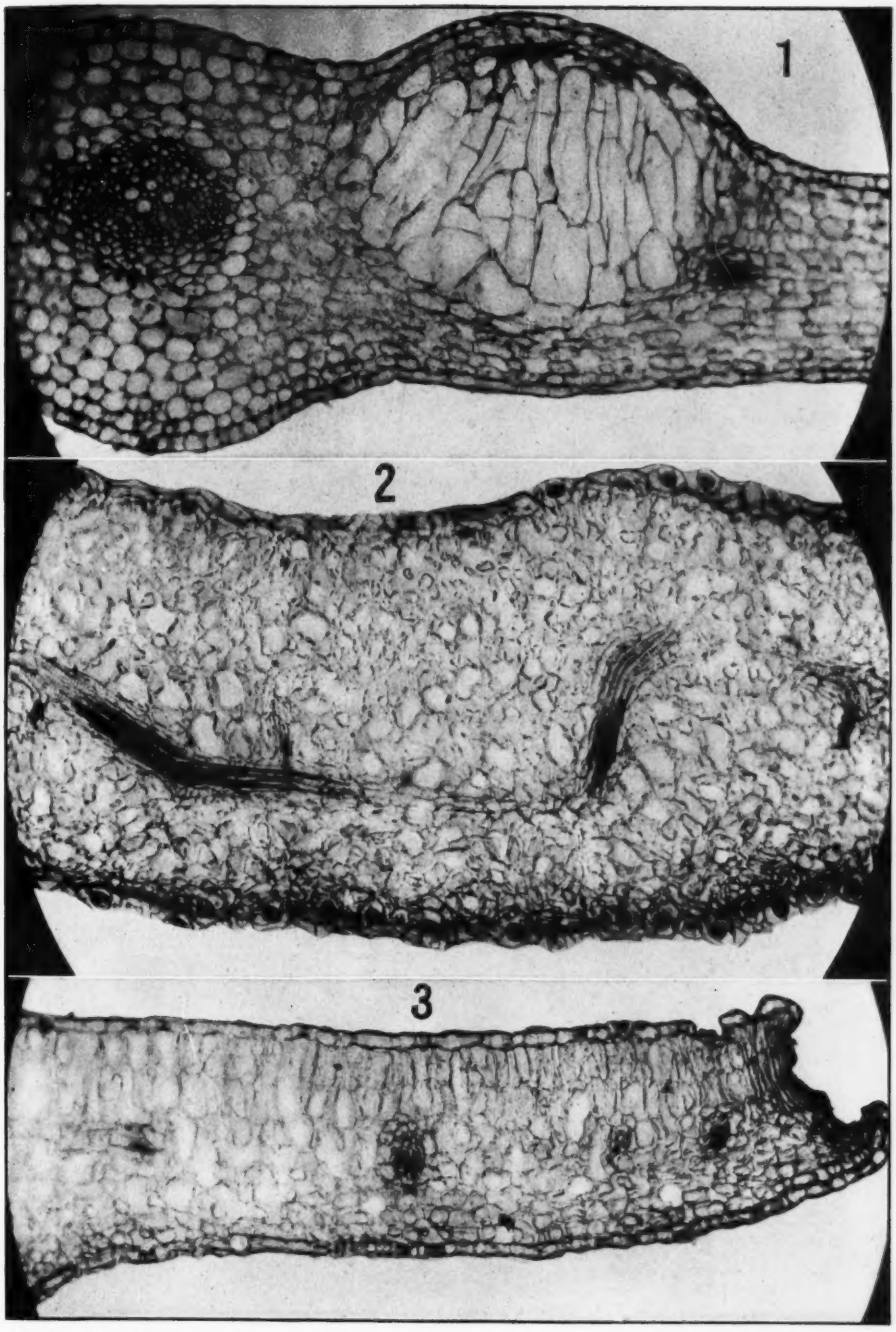
2.—Under surface of one of many leaves of a cauliflower plant exposed to vapor of primary ethylamin on December 5, and photographed December 14, slightly less than natural size. These swellings and bucklings of the parenchymatic tissue were not present when it was exposed and are possibly to be attributed to the exposure. The experiment was made by liberating the vapor from one-half gm. of primary ethylamin chlorid in 10 1/2 cubic feet of air space by adding 10 c. c. of a 15 per cent solution of sodium hydroxid. Eighteen leaves on the exposed plant showed the phenomenon, the domes of the curvatures being directed toward the lower surface of the leaf. These parts are thicker than the normal parts of the leaf and their palisade tissue has given place to round-celled parenchyma (Pl. 64, fig. 2). In a repetition of the experiment 5 leaves showed the disease on one plant and 12 on another.

PLATE 64

1.—Paraffin-embedded stained section of cauliflower leaf exposed to secondary methylamin showing hypertrophy of interior cells, absence of chlorophyll, and enlargement of the nuclei. At the left, cross section of a normal leaf rib, and at the right a longitudinal section of a smaller group of vascular bundles. Same material as Plate 46, figure 3. ×160.

2.—Cross section of cauliflower leaf (Pl. 63, fig. 2) exposed to primary ethylamin showing general thickening to double the normal thickness and absence of palisade tissue (upper surface of leaf at top.) ×160.

3.—Same leaf and section as figure 2, but from a normal portion. Palisade tissue above. ×160.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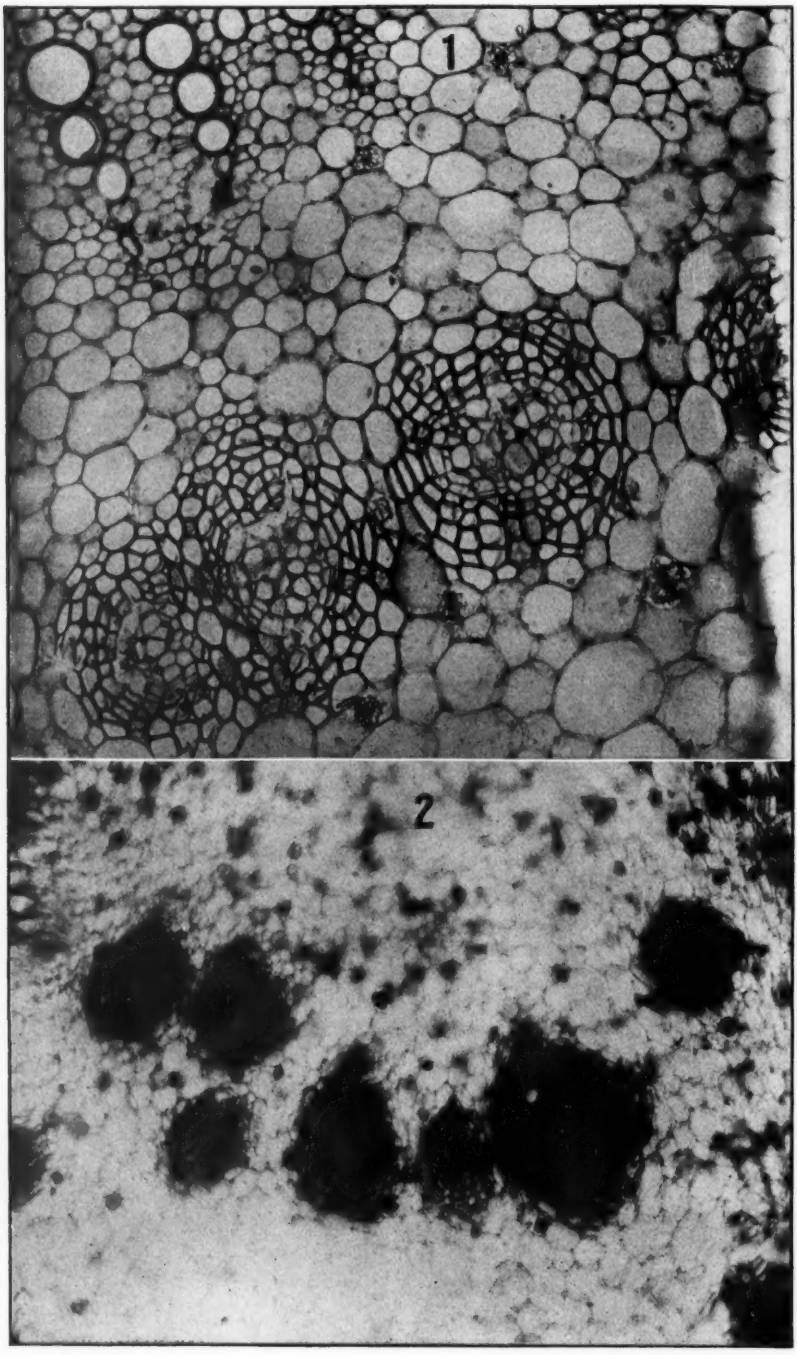
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PLATE 65

1.—Portion of ring of concentric medullary bundles in the axis of inflorescence of *Ricinus communis*. Ordinary vascular tissue at the top. Safranin stain. ×160.

2.—Group of concentric medullary bundles in a branch-gap of *Ricinus communis*. Ordinary bundles (out of focus) at top (right and left) on edges of the gap. Section is free-hand and unstained. ×75.